The Influence of Social and Genetic Relationships on Cooperation in Male Bottlenose Dolphins

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Summary

Cooperation is a ubiquitous phenomenon and a fundamental trait of all forms of life but to some degree has remained an enigma for evolutionary biologists. The key pieces of this long-standing puzzle have been identified and put together, but gaps to be filled concern how cooperative relationships are formed and maintained. Little is known about cooperative partner choice, particularly in complex societies where kin availability is low and individuals have a choice about with whom and when to cooperate.

In Shark Bay, Western Australia, bottlenose dolphins live in a remarkable society where males cooperate to compete over access to females by forming multi-level alliances in an unbound fission-fusion network. Second-order alliances, consisting of 4-14 males, are the core unit of male alliances and can last up to decades. Within second-order alliances, pairs or trios form first-order alliances to consort oestrus females. In contrast to the stable second-order alliances, first-order alliance composition can vary in different consortships.

The nested alliances in of the Shark Bay dolphins constitutes one of the most complex social systems observed outside of humans. Yet, little is known on the ontogeny of these alliances and if their formation is influenced by relatedness. Thus, our ability to compare mechanisms underlying cooperation in complex societies is limited. To fill this gap, I combined an unprecedented set of behavioural data collected over almost four decades with high resolution genomic data.

In the first part of this dissertation, I investigated when and among which males second-order alliances are formed. During adolescence, males are physically but not socially mature. In early adulthood, however, males have established second-order alliance memberships and successfully engage in consortships. To address this aim, I analysed social bonds of 59 individual male dolphins during seven years of their adolescence and adulthood, respectively. I found that most social bonds persisted across the two age stages, independent of relatedness, particularly between similarly-aged males. Overall, my findings suggest that male dolphins prefer peers over relatives as second-order alliance members and that social relationships in adolescence foreshadow alliance membership in adulthood.

In the second part, I combined long-term behavioural and genomic data to assess how genetic relatedness, social bond strength and age similarity influence the choice of allies on both alliance levels. Using our long-term behavioural data and based on 25 adult individual second-order alliance members, I retroactively identified all disregarded potential partners during the adolescence of each of these males. The availability of kin during adolescence was generally low and individuals did not prefer kin over non-kin as second-order alliance members once adults. Rather, second-order alliance

membership was best predicted by social bond strength during adolescence. Social bond strength was of particular importance when potential alliance members were younger, suggesting a preference for same-aged and older males. In a second analysis based on 53 males in six second-order alliances, I identified first-order alliance partner preferences and found that males primarily formed first-order alliances with the second-order members they shared the strongest social bonds with during the time leading up to each mating season. Therefore, partner choice on both alliance levels appears to be influenced by a male's social, but not his genealogical relationships.

That social bonds affect fitness is well documented in females but not much is known about their effect on male fitness. Owing to the importance of social bonds on male alliance formation, I investigated the effects of social attributes of 53 adult males on consortship success, which I found to be correlated with reproductive success in the third part. Males that had overall closer social bonds to their second-order alliance members and repeatedly formed first-order alliances with the same males achieved the most consortships and thus have most likely higher reproductive success. Remarkably, these social attributes were the only correlates of a male's consortship success while other variables such as home range size, age, and relatedness had no significant influence. Males that are well-connected to their second and first-order allies thus appear to gain the most fitness benefits.

In summary, the findings of this thesis provide novel evidence that although males cooperate over an indivisible resource, the benefits of cooperating with kin are likely outweighed by the direct benefits gained from cooperating with familiar, but not necessarily related partners. Similar to what is known for females in complex societies as well as for humans, male dolphins appear to invest into persisting friendships, permitting them to successfully cooperate and gain direct fitness benefits.

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“I…will confine myself to one special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory.”

– Darwin

“Alone we can do so little; together we can do so much.”

– Helen Keller

1. General Introduction

What is cooperation and why is it special?

Cooperation, defined as actions or traits that benefit others, is a fundamental trait and inherent to all forms of life. Without cooperating organelles, cellular organisms could not have evolved and without cooperating cells, the evolution of multicellular life would not have been possible. The evolution of multicellularity required multiple forms of cooperation including the inhibition of proliferation, controlled cell death, division of labour, resource allocation, and the creation and maintenance of the extracellular environment (Aktipis and Maley, 2017). Yet, this elaborate form of cooperation seems to be highly advantageous because it evolved independently several times (Knoll, 2011). While the evolution of complex, multi-cellular organisms is already striking on its own or in Darwin's words contains "endless forms most beautiful and most wonderful" (Darwin, 1859), cooperative behaviours between individuals make life truly spectacular.

Cooperative acts can be observed between members of the same species as well as between species. As humans, we are all well acquainted with both: on our free will, we place our life in someone else's hands every time we board a plane or bus, and marvel or partake in perfectly orchestrated concerts and ballets. Furthermore, our complex societies would not be possible without a high division of labour where specialists carry out different functions such as doctors looking after our health, teachers educating our children, construction workers building our shelters, and farmers and chefs producing and preparing our food. We cooperate with other species for example with dolphins to fish (Pryor and Lindbergh, 1990), with dogs to rescue people buried underneath an avalanche (Techel and Zweifel, 2013), or until recently, with horses to plough fields or for transport. However, astonishing examples of inter-individual cooperation are not limited to humans but are widely observed among all domains of life, consisting of animals, plants, fungi, and bacteria (Tables 1.1 and 1.2).

Due to its centrality in biology and its captivating nature, the study of cooperation has attracted a large number of scientists. Owing to this, a variety of terms surrounding cooperation mushroomed. Different people used different terms to denote the same phenomenon or *vice versa* used the same term to denote different phenomena (West et al., 2007). For the purpose of this thesis which revolves on male cooperation in bottlenose dolphins (*Tursiops aduncus*), it is thus important to state what I refer to as 'cooperation' and some of the crucial terms and concepts connected to it. Here, I define cooperation as an interaction that benefits the recipient(s) but not necessarily the actor performing a cooperative act (Sachs et al., 2004). Cooperative acts that solely benefit the recipient are 'altruistic' while such that are beneficial for both, actor and recipient, are 'mutualistic'. A mutualistic behaviour

does not need to be beneficial to the actor at the time of its occurrence, benefits can also be gained with a delay in time.

Cooperation - the 'special difficulty' and its ultimate explanations

Cooperation has equally fascinated and puzzled biologists since the development of evolutionary theory because at a first glance, cooperation seems incompatible with natural selection. According to natural selection, organisms have an inherent interest to promote their own chances of survival and reproduction. Competition is unavoidable because organisms produce more offspring than can survive. As a result, only the best adapted individuals mature into adulthood and reproduce. Based on evolutionary theory, intense competition and selfish behaviours should consequently be favoured by selection while cooperative acts seemingly pose an evolutionary dead end. Yet, cooperation is an abundant and fundamental trait of living organisms. Searching for answers, biologists have found multiple explanations for cooperation which can be divided into two categories based on if cooperation leads to direct or indirect fitness benefits.

Mechanisms providing direct fitness benefits

Direct fitness benefits may be gained via mutualistic acts, implying that cooperation increases the fitness of both actor and recipient. Explanatory mechanisms for mutualistic cooperation include by-product mutualism (Connor, 1995; West-Eberhard, 1975), pseudo-reciprocity (Connor, 1986), reciprocity (Trivers, 1971b), and market effects (Noë and Hammerstein, 1994, 1995). Detailed definitions and examples for these mechanisms are provided in Table 1.1.

Table 1.1. Ultimate explanations for cooperation connected to direct fitness benefits.

Mechanism	Definition	Examples
By-product mutualism (Connor, 1995; West-Eberhard, 1975)	As a by-product of a selfish behaviour, an individual generates benefits for others. In some cases, these benefits are increased by coordinated action. Cooperative acts that are based on by-product mutualism are maintained by selfish behaviours that coincidentally benefit others.	Within species: Includes phenomena like cooperative hunting due to increased hunting success of a group compared to an individual or the formation of groups to take advantage of the vigilance of others. Between species: Palm oil plantations in Indonesia represent newly generated habitats wherein ants and ferns benefit from each other's presence. Ferns provide shelter for the ants while ants protect the ferns from herbivores and disperse their seeds (Fayle et al., 2015).
Pseudo-reciprocity (Connor, 1986)	Based on an initial investment, an individual (A) gains by-product benefits based on selfish acts of others (B). Neither A nor B can cheat: without the initial investment of A, no by-products are generated by B.	Within species: In swallows (*Hirundo pyrrhonata*), the initial food call of an individual feeding on an insect swarm attracts others which start feeding on the same insect swarm (Brown et al., 1991). Since insect swarms are hard to track when alone, the initial investment of a call is rewarded by increasing the feeding efficiency of the caller. Between species Ants improve growing conditions by tending to mushrooms which they harvest at a later point (Mueller et al., 1998).
Reciprocity (Trivers, 1971b)	Individuals A and B both invest but time-lagged. Individual A behaves in a way that temporarily reduces its fitness but increases the fitness of individual B. At a later point in time, the roles of individuals A and B are reversed. Cheating is avoided by punishment or defection if the beneficial behaviour is not reciprocated.	Reciprocity is abundant in human societies and range from low cost cooperative behaviours such as donating blood or opening doors for others to highly risky acts such as rescuing strangers while endangering one's life. The occurrence of reciprocity in non-human animals is highly debated but frequently mentioned examples are reciprocal grooming in primates (Schino and Aureli, 2009b) and bats regurgitating food for hungry nest mates (Carter and Wilkinson, 2013).

Market effects (Noë and Hammerstein, 1994, 1995)	Similar to trade between merchants, cooperation can be understood as exchange of services or resources, so-called 'commodities'. This exchange is connected to certain costs and benefits that influence the propensity of cooperation to take place between individuals. Depending on whom an individual chooses to cooperate with, different costs and benefits are experienced and thus partner choice is a crucial element. Individuals tend to choose the most competent partners (Chapais, 2006) and discontinue cooperation with unhelpful or exploitive partners (Bshary and Noë, 2003). Mechanisms promoting successful cooperative partner choice include the detection or sanction of individuals who fail to provide benefits as well as the identification of partners providing the most benefits and their successful recruitment (Melis et al., 2006). In humans, this economic representation of relationships has been linked to neurobiological mechanisms maintaining interactions with generous and social partners (Heijne et al., 2018).	<u>Within species:</u> In some primate species, subordinate individuals derive rank-related benefits by grooming higher-ranking individuals and compete over the opportunity to groom them (Schino, 2001; Tiddi et al., 2012). Thus, grooming opportunities are subject to supply and demand. <u>Between species:</u> Reef fish cooperate with cleaner wrasses (*Labroides dimidiatus*) to rid them of ectoparasites. Cleaner wrasses benefit from this interaction by getting access to a meal. Client fishes with large home ranges switch or punish cheating cleaner wrasses that feed on mucus or gills instead of ectoparasites. Clients with smaller home ranges do not have the possibility to switch partners but instead, build relationships before an interaction takes place. Partner choice is thus a pivotal element of the cleaner-client interaction (Bshary and Noë, 2003).

Ultimate explanations for cooperation increasing an actor's direct fitness benefits. Examples for each mechanism are provided in the last column.

To assign a cooperative behaviour to one of the mechanisms it is crucial to understand their differences and similarities (Fig. 1.1). By-product mutualism and pseudo-reciprocity are similar in that both mechanisms generate benefits to others based on selfish behaviours by the actor. However, unlike by-product mutualism, pseudo-reciprocity requires an initial investment which is costly to the actor. A costly initial investment is common to pseudo-reciprocity and reciprocity. In contrast to pseudo-reciprocity though, reciprocity is not based on a selfish behaviour but consists of a time-lagged exchange of costly behaviours, which are not necessarily the same. Both reciprocity and market effects involve an exchange of resources. However, in contrast to reciprocity, market effects provide a nearly

simultaneous benefit for both parties because they involve a trade of resources. Moreover, in market effects individuals choose among a pool of potential partners, aiming to outbid others in order to be chosen as cooperative partner. Consequently, the response to the initial investment fluctuates and is dependent on supply and demand (Schino and Aureli, 2010).

Compared to mutualism and pseudo-reciprocity where individuals act selfishly and the short term exchange of resources within a 'biological market', reciprocity is thought to be cognitively more demanding since multiple conditions need to be met for it to occur: individuals need to have the opportunity to interact more than once, recognise each other, and be able to keep track of their previous interactions. Owing to this complexity, other mechanisms than reciprocity are the most parsimonious explanations for the majority of cooperative behaviours that generate direct fitness benefits. Many examples for reciprocity that are described in text books and publications had to be revised since they could also be explained by alternative, comparatively simpler mechanisms. The regurgitation of vampire bats (*Desmodus rotundus)* to hungry roost mates, a frequently mentioned example, could also be caused by hungry individuals harassing individuals that returned from hunts successfully (but see Carter and Wilkinson, 2013). Similarly, 'reciprocal' grooming can often be explained by kin selection or other purposes such as strengthening social bonds which in turn, facilitates social interactions that are connected to fitness benefits (Silk, 2014). Hence, caution should be applied before attributing a cooperative behaviour to reciprocity.

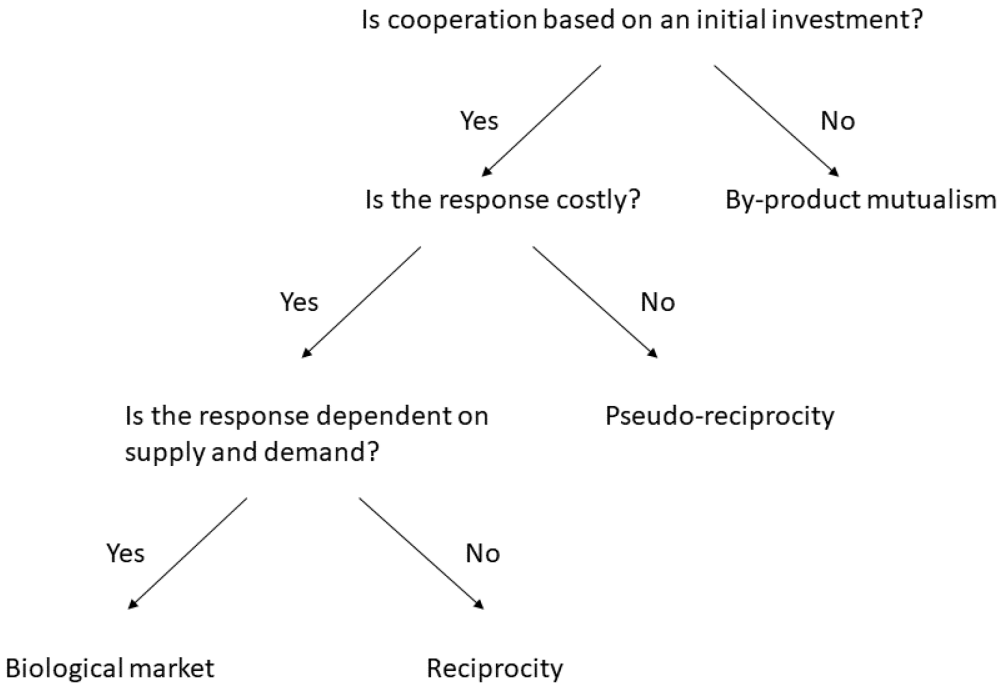

Figure 1.1. Flow chart depicturing the differences between cooperative mechanisms providing direct fitness benefits to the actor (modified and expanded from Bergmüller et al., 2007).

Mechanisms providing indirect fitness benefits

Indirect fitness benefits are acquired when cooperation increases the reproductive success of an actor's relatives but not the actor itself (Hamilton, 1964). Indirect fitness benefits are the only evolutionary stable explanation of altruistic behaviours. Kin-biased cooperation can be positively selected for because relatives share genes, permitting the indirect transfer of genetic material to the next generation through the reproduction of relatives. The strategy to cooperate with kin over non-kin is referred to as 'kin selection' (Table 1.2). The principle of kin selection is contained in an elegant and remarkably simple inequation known as Hamilton's rule. Hamilton's rule predicts that cooperation is positively selected for when the costs to the actor ('c') are smaller than the product of the fitness benefits gained by the recipient of a cooperative act ('b') and the genetic relatedness between the actor and recipient ('r'): $rb > c$.

According to Hamilton's rule, individuals achieve a higher overall fitness, *i.e.*, inclusive fitness, by cooperating with relatives when the loss of direct fitness benefits (c) is smaller than the indirect benefits gained (rb). Thus, individuals generally gain more fitness benefits by cooperating with kin compared to non-kin. Hamilton's rule has been proven to be a powerful explanation for the enigma of cooperation because kin-biased cooperation is highly prevalent (Smith, 2014).

The propensity to cooperate with kin over non-kin is facilitated by kin discrimination or population viscosity (Hamilton, 1964). Kin discrimination enables individuals to reliably recognise kin based on phenotypic cues while a low population viscosity keeps relatives in each other's vicinity. If population viscosity is low, individuals can indiscriminately cooperate within their social group because group members are expected to be kin. Kin selection can therefore also occur in absence of a mechanism to distinguish kin from non-kin.

Another, mechanism to gain indirect benefits is the 'green-beard' effect (Hamilton, 1964). Here, a shared cooperative gene is manifested in a clearly recognisable manner, allowing carriers of the gene to identify and cooperate with each other while avoiding cooperation with uncooperative non-carriers (Table 1.2). Initially introduced by Hamilton as a thought experiment, Dawkins' named the green-beard effect in his book *The Selfish Gene* (1976) where he used a green beard as an example for the phenotypic manifestation of the cooperative gene ("*I have a green beard and I will be altruistic to anyone else with green beard*"). Both authors made the point that indirect fitness benefits can also be gained when cooperating entities are not genealogical kin (Gardner, 2019). Independent of whether or not they believed in their existence, advances in the molecular tool set of geneticists led to the detection of green-beard effects in the natural world (Table 1.2).

Although examples are available for both mechanisms with which indirect fitness benefits can be gained, they differ substantially in the number of supporting examples. A large number of studies found individuals to preferably cooperate with kin over non-kin in a wide range of taxonomic classes but only a few studies found evidence for a green-beard effect, mainly in microbes (Gardner, 2019). The green-beard effect requires the presence of a gene (or cluster of linked genes) encoding two phenotypes: one to encode a cooperative behaviour and a second one to express the green beard. If these two become unlinked, false green beards appear in the population. Since false green beards enjoy the benefits of cooperation but do not pay the costs, they have an advantage over the true green beards, eventually leading to the extinction of the latter. Hence, green-beard effects are more common in clonally reproducing species while sexually reproducing species seem to gain indirect fitness benefits from cooperating with kin either via kin discrimination or low population viscosity. My study subject, as well as most studies I refer to, concern sexually reproducing animals. Green-beard effects are mentioned here for the sake of completeness but from here onward, wherever I refer to indirect fitness benefits it indicates fitness benefits gained from kin-biased cooperation.

Table 1.2. Ultimate explanations for cooperation connected to indirect fitness benefits.

Mechanism	Definition	Examples
Kin selection / Inclusive fitness (Hamilton, 1964)	Individual A behaves in a selfless manner and thereby increases the reproductive success of individual B which is a relative of A.	In turkeys (*Meleagris gallopavo*), two males consisting of a dominant male and a related but younger subordinate, jointly carry out courtship displays. Only the dominant male mates with the female while his relative gains indirect fitness benefits (Krakauer, 2005). In sea rockets (*Cakile edentula*), sibling plants sharing the same pot do not compete for soil nutrients by root growth. Unrelated plants in contrast, compete (Dudley and File, 2007).
Green-beard effect (Hamilton, 1964)	Individuals cooperate based on a cooperative gene. The gene leads to the expression of a clearly identifiable trait (e.g., a green beard). This trait is recognised by others and carriers selectively cooperate with each other.	Amoebae (*Dictyostelium discoideum*) carrying the *csa*-gene variant form fruiting bodies with other carriers but exclude similar looking non-carriers (Queller et al., 2003). The tumor-inducing (Ti) plasmid from the plant pathogen *Agrobacterium tumefaciens* provokes gall formation and the synthesis of opines in plants which can only be accessed as energy source by Ti-carrying bacteria (White and Winans, 2007).

Ultimate explanations for cooperation based on an increase in indirect fitness benefits. Examples for both mechanisms are provided in the last column.

The proximate level: cooperative partner choice and ontogenetic aspects of cooperation

The ultimate mechanisms introduced in the previous section explain the adaptive value, *i.e.,* the gain of fitness benefits, of cooperation. However, for an integrative comprehension of a behaviour, its proximate mechanisms need to be understood as well (Tinbergen, 1963). The proximate level includes questions on the ontogeny of a behaviour, referring to how a behaviour develops across an individual's lifespan as well as over the evolutionary history of a species, and the external and internal stimuli evoking the behaviour. To piece together the puzzle of cooperation we therefore need to identify the

adaptive benefits of cooperation on the ultimate level and in addition, investigate when and among whom cooperative acts occur and how cooperative relationships are formed to gain insights into the proximate causes.

Who cooperates?

For cooperation to evolve and to be maintained it has to be connected to fitness. Therefore, cooperative acts should be directed towards individuals from which direct or indirect fitness benefits can be gained. As a consequence, cooperation often takes place between relatives (Silk, 2014; Smith, 2014), indicating that individuals choose their partners as predicted by Hamilton's rule. Aside from genetic similarity, traits in which cooperating individuals were found to be similar include age and rank (Mitani et al., 2002b), tool use (Bizzozzero et al., 2019), and personality (Massen and Koski, 2014). The benefits of cooperating with a self-similar partner may be attributed to facilitated coordination and cooperation due to shared interests or needs (Ruckstuhl and Neuhaus, 2001). The choice of cooperative partner choice can thus be induced by homophily of a certain trait. An assumption which is supported by multiple studies (Antal et al., 2009; Fu et al., 2012; McPherson et al., 2001).

In group-living mammals, cooperative partner choice is often influenced by social bonds. Social bonds can be defined as affiliative and persisting relationships, sometimes referred to as 'friendships' (Cords and Thompson, 2017; Massen, 2017; Silk, 2002). In wild chimpanzees (*Pan troglodytes*), for example, social bonds facilitated cooperative sharing of food more than harassment or trade (Samuni et al., 2018). The propensity to recurringly cooperate with social partners could be caused by increased levels of trust to a closely bonded partner (Ames et al., 2006; Engelmann and Herrmann, 2016) and higher levels of familiarity with each other's behavioural responses which potentially increase the ability to coordinate behaviours as observed in various species (Keller et al., 2017; Prior et al., 2019; Riley et al., 2019).

Recent work showed that social bonds generate multiple adaptive fitness benefits which may or may not be linked to cooperation. Fitness benefits include increased reproductive output (e.g., feral horses *Equus ferus*, Cameron et al., 2009; chimpanzees, Langergraber et al., 2013; Assamese macaques *Macaca assamensis*, Schülke et al., 2010; house mice *Mus musculus*, Weidt et al., 2008), life span (e.g., savannah baboons *Papio cynocephalus*, Silk et al., 2010b), infant survival (e.g., savannah baboons, Silk et al., 2003; Silk et al., 2009), health (Capitanio et al., 1998), and decreased mortality (e.g., humans, Holt-Lunstad et al., 2010).

The effects of relatedness and social bonds on cooperative partner choice are often difficult to disentangle because relatives are often preferred social partners (Carter et al., 2019). This suggests that where possible, species have evolved to allow individuals to maximise their inclusive fitness.

Alternatively, cooperative relationships could also have initially evolved to provide indirect fitness benefits. Over time, however, persisting cooperative relationships were selected to be extended to non-kin because individuals gained direct fitness benefits through the formation of social bonds. Particularly in monotocous species with slow life histories and large interbirth intervals, demographic constraints may not allow for the formation of kin-biased social bonds (Faaborg et al., 1995; Heinsohn, 1991; Mitani et al., 2002b). In such species, individuals might either form social bonds irrespective of relatedness or choose non-kin whenever kin is not available. Unfortunately, the crucial element of kin availability is often neglected, most likely because long-term behavioural and/or genetic data are missing, especially in species with slow life histories.

Ontogenetic aspects of cooperative relationships

Kinship, homophily, and social bonds can explain among which individuals cooperative relationships occur in a vast number of cases. However, where cooperation is not based on kin, relatively little is known about under which circumstances relationships are formed and how they are maintained (Alberts, 2019; Amici et al., 2019).

In cooperative breeders and eusocial insects, where there is a clear division of labour between helpers and breeders, cooperative relationships are based on an individual's role within the social group (Dey et al., 2013). For instance, the role that honey bees (*Apis mellifera*) have within the hive and with whom they cooperate is age-dependent. Younger bees tend to the nest while older ones forage. Role changes were shown to be regulated by neuromodulators (Schulz and Robinson, 2001) and the age demography of the colony (Huang and Robinson, 1996), thereby suggesting that individual behaviour does not affect a bee's role within the hive.

The ontogeny of cooperative relationships is less clear in complex mammalian societies. There, individuals forge social bonds to some group members but not to others. In some species, such as cercopithecine primates or killer whales (*Orcinus orca*), social bonds are formed within matrilines (Bigg et al., 1990; Silk et al., 2010a). Thus, simple rules based on familiarity can explain the formation of social bonds. However, it is less clear how social bonds develop in open fission-fusion societies with their innumerable options to cooperate or compete with others. As a result, individuals are forced to choose whom to cooperate with in a 'biological market' and to forge social bonds to desirable cooperative partners.

Recent research has shown that social bonds between cooperative partners develop before they become important in terms of providing adaptive benefits through joint cooperation. In macaques (*Macaca* spp.) where females cooperate and form social bonds within matrilines, females forged strong social bonds to their maternal relatives already as immatures (Amici et al., 2019). Similarly, in

chimpanzees, living live in open fission-fusion societies and where male-male social bonds facilitate cooperative behaviour in adulthood, adolescent males did not differ in the number of social bonds compared to adult males (Sandel et al., 2020). These studies suggest that social bonds in complex societies are not formed on an *ad-hoc* basis, but that individuals invest into their future cooperative partners from an early age onward. Since selection can only act on the phenotype, it appears likely that traits influencing an individual's ability to forge social bonds to others are likely to provide benefits also in early life. In the case of young male chimpanzees, social bonds to older males are probably beneficial because older males can offer coalitionary support and provide protection from competitive, prime-aged males (Sandel et al., 2020).

Cooperating to compete: The conundrum of male cooperation

Theoretical and empirical research have increased our understanding of the ultimate and proximate causes of cooperation. These advances now allow us to investigate the particularly puzzling phenomenon of male cooperation. Male cooperation is an evolutionary conundrum because males are mostly limited in their reproductive success by access to females, an indivisible resource. In species with skewed operational sex ratios, indicating a low availability of fertile females compared to the number of sexually active males, males compete over females. In contrast to males, the reproductive success of females is limited by access to food and safety which can be shared. It is thus not surprising that in the majority of group-living mammals females form persisting cooperative relationships while males engage in one-off coalitions to attain a higher rank in order to increase access to females (e.g., savannah baboons, Noë and Sluijter, 1995) or other short-term cooperative acts such as preventing territorial takeovers by other males (e.g*.,* hyaenas *Hyaena hyaena*, Caro, 1994; Wagner et al., 2008). However, in some species, primarily in mammals and birds, males form long-lasting social bonds which are connected to adaptive benefits. In Assamese macaques, for example, social bonds among unrelated males predicted coalition formation, which in turn, influenced future dominance and thus reproductive success (Schülke et al., 2010).

Groups of two or more males who repeatedly cooperate to compete with others over access to females are known as alliances (Harcourt and de Waal, 1992). Alliances occur in humans (Chagnon, 1988), non-human primates (e.g., chimpanzees, Mitani et al., 2002a; savannah baboons, Noë, 1992), lions (*Panthera leo*, Packer et al., 1991), cheetahs (*Acinonyx jubatus*, Caro, 1994), lekking birds (e.g*.,* long-tailed manakins *Chiroxiphia linearis*, McDonald and Potts, 1994) and bottlenose dolphins (Connor et al., 1992). Since allied males cooperate to gain access to females but not all males succeed in siring offspring, kin selection would the most parsimonious explanation for the evolution and maintenance of male alliances.

Kin selection explained patterns of alliance formation in cheetahs but surprisingly, was not supported in the other species. Rather than cooperating with kin, male lekking birds, for example, employ a queuing system where young males start at the bottom of the hierarchy and rise towards the top as they age (McDonald and Potts, 1994). In lions, paternity concentration and the tendency to form coalitions with non-kin decreased with alliance size (Packer et al., 1991). Lions therefore appear to employ different strategies depending on alliance size. In dolphins, a male's propensity to form alliances with kin could be habitat or population-specific. This is because in some populations, levels of relatedness among allies were higher compared to the population average (Diaz-Aguirre et al., 2018; Parsons et al., 2003), but this pattern was not consistent across all populations (Krützen et al.,

2003; Möller et al., 2001; Wells, 2014). Similarly, no consistent pattern to cooperate with kin over non-kin was observed in male chimpanzees and humans (Langergraber et al., 2009; Macfarlan et al., 2014; Mitani et al., 2000; Nolin, 2011).

Generally, kin selection has been proven to be a powerful explanation for cooperation (Smith, 2014). Nevertheless, male alliances do not necessarily need to follow this predictions when alliance formation increases a male's direct fitness. Allied males cooperate to increase access to females. Hence, a male's reproductive success is not only determined by his own traits but also by the traits and commodities of his alliance partners. While indirect fitness benefits can be gained by cooperating with relatives, choosing a valuable, but unrelated partner might prove to be advantageous if such a partner exhibits certain traits, including resource holding potential (Parker, 1974), cooperativeness (Heinsohn and Packer, 1995) and competence (Chapais, 2006). Given that alliance formation increases a male's competitive potential compared to when acting alone (Díaz-Muñoz et al., 2014), choosing the wrong allies could prove costly if this results in a significant decrease in reproductive success (Bshary and Grutter, 2005; Connor and Mann, 2006). Yet, relatively little is known about the ontogeny of male alliance formation. Furthermore, in species where alliances are formed among non-kin, the criteria based on which males choose their alliance partners are unknown. A crucial point to understanding the formation of male alliances is thus to investigate the correlates predicting alliance formation.

The Shark Bay bottlenose dolphins

Study site and behavioural data collection

The Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) population off Monkey Mia in the Eastern gulf of Shark Bay, Western Australia (Fig. 1.2), is among the world's best-studied dolphin populations. Systematic behavioural data collection began in 1984 and has been ongoing ever since. Behavioural observations are collected in the form of 'surveys', where we use boats to travel among groups of dolphins. During surveys, we record group composition using photo ID (Würsig and Würsig, 1977) and the 10m chain rule (Smolker et al., 1992), group activity, location, depth, water temperature and other data (Connor and Krützen, 2015). Group composition data from surveys led to the detection of male alliances already during the 1980s. Since then, the male alliances in Shark Bay have been extensively studied, totalling to close to 40 years of observational data on male alliance behaviour (Connor and Krützen, 2015).

Over these four decades, it became clear that male bottlenose dolphins in Shark Bay exhibit one of the most remarkable male alliance systems known, matched in their complexity only by human societies (Connor and Krützen, 2015). The dolphin alliance system is unusual because male dolphins exhibit long-term, multi-level alliances with context-dependent interactions in an unbound fission-fusion society (Connor et al., 2017; Connor et al., 2000b; Randić et al., 2012). Even though male dolphins have been reported to form a..iances in other populations (Owen et al., 2002; Parsons et al., 2003; Wiszniewski et al., 2012a), the Shark Bay population is unique in that it appears to be the only population where up to three levels of nested alliances have been documented to date (Connor and Krützen, 2015; Randić et al., 2012).

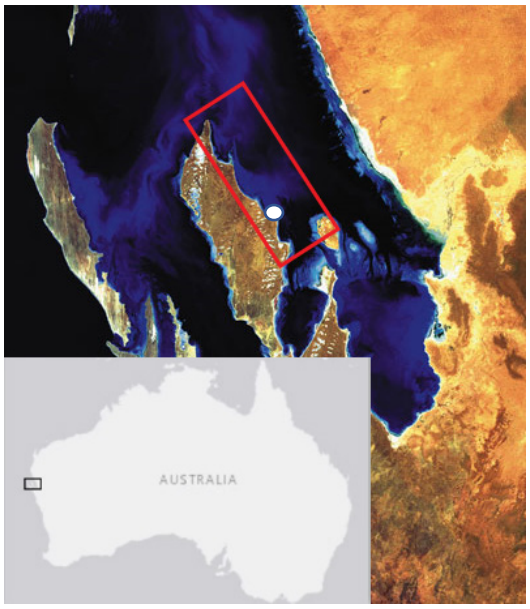

Figure 1.2. Map of the study site in the Eastern gulf of Shark Bay, Western Australia. The area within the red rectangle denotes the current study area. The white dot indicates Monkey Mia, where researchers started to observe wild bottlenose dolphins in the early 1980s (modified from sharkbay.org and Connor et al., 2017).

The complex male alliance system in Shark Bay

Every decade of dolphin research in Shark Bay was connected with a hallmark discovery on its complex male alliance system. During the 1980s, behavioural observations in a small area off Monkey Mia revealed strong, year-round associations between two to three males ('alliances') which seasonally cooperated to sequester and control movements of single oestrus females within so-called 'consortships' (Smolker et al., 1992). From 1987 onward, particular alliances were found to jointly steal females from other alliances or to defend against such attacks (Connor et al., 1992). This suggested a multi-level alliance structure where pairs or trios form 'first-order alliances' which are themselves nested within 'second-order alliances'. The expansion of the study site in the early 1990s resulted in the detection of a much larger 'super-alliance' consisting of 14 males. In contrast to the small second-order alliances consisting of two stable first-order alliances, members of the super-alliance formed labile first-order alliances, changing in composition over different consortships. In the early 2000s, when the study site was expanded to its current size, it became clear that this is not owed to a dichotomy in alliance tactics. Rather, more extensive research revealed that second-order alliances follow a continuum of different sizes, ranging between 4-14 members (Connor and Krützen, 2015; Connor et al., 2011). Moreover, it turned out that first-order alliances are more labile than second-order alliances, although some males have marked first-order alliance partner preferences. Second-order alliances can persist for multiple decades after they are initially formed and are therefore nowadays recognised as the core alliance unit in Shark Bay (Connor and Krützen, 2015). Data collected on multiple second-order alliances over the last decade suggested that male dolphins even form third-order alliances, appearing to serve the same function as second-order alliances, *i.e.,* aiding in the defence and stealing of females (Connor and Krützen, 2015).

Male alliances in dolphins differ from the purely reproductive alliances or coalitions observed in other species (Díaz-Muñoz et al., 2014; Silk, 2002; Thompson, 2019) in that allied adult male dolphins exhibit high levels of association year-round (Smolker et al., 1992) and are characterised by increased rates of affiliative behaviours such as petting where dolphins touch and stroke each other with their pectoral fin (Connor et al., 2006; Connor et al., 2000b). The year-round association patterns and thus familiarity with each other's behavioural responses might serve to increase the males' coordinative abilities. During consortships, males engage in remarkable levels of motor and acoustic coordination, including synchronous surfacing and the synchronised production of threat vocalisations (Connor et al., 2006; Moore et al., 2020).

Genetic sampling and insights from earlier genetic studies

Genetic sampling of the Shark Bay dolphins started in 1997 (Krützen et al., 2002) and yielded more information on the underlying population structure and provided valuable insights into unique behavioural characteristics. The usage of marine sponges as foraging tool, for example, was found to be a cultural trait transmitted through the maternal line (Krützen et al., 2005).

Although already suspected based on male behaviour during consortships (Connor et al., 1996), genetic methods revealed that alliance membership is indeed linked to direct fitness since non-allied males sired very few, if any, offspring (Krützen et al., 2004a). The same study found that paternities are skewed within first-order alliances. This suggests that not all alliance partners are equally successful, even though all males presumably mate with their consort. A later study found that males which repeatedly formed the same first-order alliance within the network of their second-order alliance, *i.e.,* such with high first-order alliance stabilities, achieved a higher consortship rate (number of days in consortships/number of days observed) compared to males with low first-order alliance stabilities (Connor and Krützen, 2015). In case consortship success predicts reproductive success, reproduction is hence, also skewed within second-order alliances. More recently, it became evident that consortship success not only differs within but also between second-order alliances (Connor et al., 2017). Second-order alliances in the North of the study site consort females at a higher rate compared to the South. The price for this success, however, are higher levels of aggression since marking from tooth rakes are greatest in the North (Hamilton et al., 2019). The more intense competition in the North of the study site might also explain the propensity of males in the North to consort in trios compared to pairs in the South (Connor et al., 2017).

Earlier studies have also addressed whether alliance formation in Shark Bay is kin-biased and thus linked to indirect fitness benefits. In contrast to the large 'super-alliance', average relatedness was higher than expected by chance in the small second-order alliances consisting of 4-6 males detected in the early 1990s (Krützen et al., 2003). However, the alliances investigated in this study represent the extremes in terms of second-order alliance size. Data on more second-order alliances, particularly such of intermediate size, are needed to investigate the influence of relatedness on male alliance formation.

By assessing the genetic population structure, it became clear that there is limited dispersal of either sex (Krützen et al., 2004b), leading to a high number of inbred matings (Frère et al., 2010a). Theoretically, this bisexual philopatry also enables individuals of both sexes to form persisting social bonds with relatives. Although it is known that male calves and juveniles (two years pre- and two years post-weaning, respectively) invest more time into social activities compared to females of the same

age (Krzyszczyk et al., 2017; Stanton et al., 2011), the long-term persistence of early dyadic associations in males, for example whether social bonds formed in early life foreshadow alliance membership and whether they are biased towards kin, has never been formally tested. The fact that the ontogeny of male alliance formation has not been investigated yet is due to the slow life history of dolphins. Male dolphins reach sexual maturity around the age of twelve (Kemper et al., 2014). However, social maturity is reached even later. Dolphins start to successfully consort in their mid-teens (Connor and Krützen, 2015) and reach their reproductive peak presumably in their early twenties (Horlacher, 2018). Seeking answers on the ontogeny of male alliances thus requires long-term behavioural data.

Considerations to make when investigating the effect of relatedness on polyadic cooperation in the absence of pedigree data

Demographic constraints and individual kin availability

In some species, individuals might prefer relatives as alliance partners that are simply not available demographic constraints. Especially In situations where kin availability is low but alliance formation is essential for reproductive success, males may make the best-of-a-bad-job by forming alliances with non-kin out of necessity. To fully understand male cooperation, it is therefore pivotal to investigate for the availability of kin on an individual level.

Average relatedness of groups vs. dyadic relatedness

Research on multiple dolphin populations revealed that first-order alliances in Coffin Bay, South Australia, and in the Bahamas (Diaz-Aguirre et al., 2018; Parsons et al., 2003) consisted of males more closely related than expected by chance but this was not the case in Port Stephens, South Australia, and Sarasota Bay, Florida (Möller et al., 2001; Owen et al., 2002). All these studies, including the one in Shark Bay (Krützen et al., 2003), assessed the potential effect of kinship on alliance formation based on estimates of average relatedness within alliances relative to a population mean. While this approach might be appropriate where alliances consist of litter mates, it most likely omits critical information on the dyadic level where this is not the case. This is because alliances between non-litter mates consist of males with a more diverse genetic background.

Alliances containing males of different levels of relatedness are likely to occur in monotocous species where maternal half-siblings are expected to be scarce. This effect is even more pronounced where paternity concentration is low and thus peers are rarely half-siblings. Considering that at least in female mammals, complex social relationships mostly occur in species where average relatedness among group members is low (Lukas and Clutton-Brock, 2018), it is crucial to measure relatedness on the dyadic and not the group level to infer its effect on alliance formation. Relatedness might be negligible in the formation of larger group but play a role when smaller cooperative entities are formed within these groups. In the Shark Bay alliance system, for example, second-order alliances might consist of mostly unrelated males but males might prefer relatives as first-order alliance partners.

Pairwise relatedness estimates

Relatedness between individuals can be estimated using genetic tools in the absence of pedigrees. However, this has some inherent methodological issues which need to be taken into consideration

when pursuing such an approach. Pairwise relatedness estimates gained from genetic techniques are assumed to truly reflect the proportion of shared to non-shared nucleotide sequences that are identical by descent. However, most studies only genotype a subset of the population at a low number of highly variable microsatellite loci, usually ranging between 10 and 20 (Guichoux et al., 2011). This approach bears three potential sources leading to erroneous relatedness values. The first one pertains to choosing the most suited method to generate relatedness values. This choice is related to precision, *i.e.*, how accurately the estimated relatedness values reflect the true values as well as any systematic biases. The second concerns the inclusion of a sufficient number of genetic markers to achieve small standard deviations between values of the same relationship category to correctly identify a dyad's relationship. Third, erroneous relatedness values can arise where the estimation of population allele frequencies is erroneous because of small number of genotyped individuals or spatial substructure is unaccounted for.

Over the last decades, different methods have been developed to estimate relatedness from genetic data of which none consistently outperforms the others (Csillery et al., 2006). Hence, it is crucial to evaluate different methods and to choose the one most suited for one's study population. One way to achieve this is by simulations. Based on a population's allele frequencies, dyads of known relationships, for example half-siblings, are simulated. The relatedness estimator showing the smallest standard deviation and highest correlation with the true values should be used for analyses (Wang, 2011). Simulation studies can also be used to assess marker performance. If relatedness values for a certain relationship are scattered over a wide range, the performance of the genetic markers is low. A narrow range suggests that that relatedness estimates from the chosen markers reliably reflects genealogical relatedness.

Genetic marker systems to estimate relatedness

To date, the majority of genetic work in wild populations was carried out by either sequencing short stretches of mitochondrial DNA (mtDNA) or scoring autosomal microsatellite loci. Mitochondrial DNA is haploid, inherited through the maternal line, and is characterised by an elevated mutation rate compared to nuclear DNA (Brown et al., 1979; Lynch, 2007). These qualities make mtDNA an excellent tool to study a species' demography and social structure, as well as to infer maternal relatedness to some degree. Microsatellites, repetitive DNA motifs of 1-6 bp (Tautz, 1989), are highly abundant and distributed all over an organism's genome. Microsatellites are characterised by high genetic diversity owing to their high mutation rate (Schlötterer, 2000) and thus a high per locus information content. The distribution of microsatellites across the genome allows the combination of multiple microsatellites, increasing analytical power without inducing any issues of linkage disequilibrium. Until

recently, high costs were involved when developing microsatellite panels and genotyping of microsatellites is still comparatively expensive. Most studies therefore target a relatively small number of microsatellites (Guichoux et al., 2011). Although this relatively low number of microsatellites contains reliable information to infer paternities (Harrison et al., 2013), it has been shown to be of limited power to resolve more distant genetic relationships (Attard et al., 2018; Städele and Vigilant, 2016; Taylor, 2015).

Compared to microsatellites, single nucleotide polymorphisms (SNPs) have a lower per locus information content because they are mostly bi-allelic. However, SNPs are more abundant in the genome. With sequencing costs dropping and efficiency increasing using next-generation-sequencing (NGS) technology, thousands of SNPs can now be genotyped with relatively little effort at small costs. Although thousands of microsatellites can also be genotyped using NGS as we demonstrated in humpback dolphins (*Sousa sahluensis,* Kedzierska et al., 2018), several approaches targeting SNPs have been developed without requiring prior information on a species' genome. Such genotyping-by-sequencing approaches can therefore, also be applied to non-model organisms (Narum et al., 2013).

Reduced representation library approaches, where the same fraction of the genome is sequenced in multiple individuals, have surged in popularity over the last years (Andrews et al., 2016). One such approach is double digest restriction-site associated DNA sequencing (ddRAD, Peterson et al., 2012). Using two restriction enzymes, genomic DNA is digested. To obtain a subset of the genome, the fragmented DNA is size-selected DNA and subsequently sequenced. Sequencing typically yields several thousands of SNPs and was found to yield more accurate coefficients of relatedness and to resolve genetic relationships to a higher degree compared to microsatellites (Lemopoulos et al., 2019; Thrasher et al., 2018).

Precise relatedness estimates are required to investigate if kin selection underlies alliance formation or cooperative partner choice. The development of additional microsatellite markers is comparatively more expensive than the establishment of a ddRAD or another GBS approach. In addition, ddRAD approaches are less time and material consuming compared to traditional laboratory protocols targeting microsatellites. Thus, ddRAD approaches are well-suited to target questions related to indirect fitness benefits in a large number of species.

Aims of the dissertation

By studying ultimate and proximate aspects of male cooperation in dolphins, I aim to expand our knowledge of the evolution and maintenance of male alliances in Shark Bay dolphins. For this, I investigated several key aspects. On the ultimate level, I was interested in the adaptive fitness benefits of alliance formation in male bottlenose dolphins. In particular, I investigated whether individual males preferred kin over non-kin as alliance partners on both alliance levels. With this, I hoped to elucidate whether male alliance formation may yield indirect or direct fitness benefits for participating males. I further advanced on previous work by applying an individual-level instead of a group-level approach allowing me to infer kin availability. Owing to the slow life history and no apparent male dominance hierarchies, I expect relatives on the level of maternal and paternal half-siblings to be rare. In addition, the polygynandrous mating system is likely to lead to incongruent set of relatives. I therefore hypothesise the influence of kin selection on male alliances in the Shark Bay bottlenose dolphins to be highly limited and that cooperation within alliances underlies a different ultimate mechanism enhancing direct fitness of allies. To assess levels of relatedness more accurately compared to the up to 27 microsatellite markers in earlier studies, I refined a ddRAD approach and adapted it for use in bottlenose dolphins.

On the proximate level, I studied the ontogeny of male alliances by seeking answers to questions concerning their formation by investigating traits of chosen and non-chosen partners on both alliance levels. In particular, I was interested whether second-order alliance membership is already foreshadowed by patterns of associations during adolescence. By forging social bonds to future allies early in life, males create the opportunity to identify valuable partners and get familiar with each other's behavioural response and increase their levels of coordination. I also investigated whether the choice of first-order partners as adult members of second-order alliances was influenced by social bond strength to other members. While I assume that familiarity and coordination enhance consortship success, males might also bias first-order alliance partner choice to the small number of relatives available and thus aim to maximise inclusive fitness. My final aim was to elucidate how social bonds contribute to a male's reproductive success. I predicted social bonds to be advantageous in that well-connected males are likely to be chosen as first-order alliance partners and therefore have higher consortship success.

Addressing all these questions in bottlenose dolphins was only possible due to long-term behavioural data and genetic sample collection. I found myself in the fortunate position to have access to behavioural data spanning close to 40 years and an abundance of tissue samples, allowing me to gain genetic information on over 1'000 individuals. This amount of data permitted me to address these

novel questions on male alliance behaviours in a well-known population and thus to build upon a strong foundation of previous research.

Due to their complex social system, the results produced within the frame of this dissertation, significantly enhance our knowledge on the evolutionary mechanisms acting on cooperation in one of the most complex cooperative societies outside humans (Connor and Krützen, 2015). In humans, cooperation occurs largely independent of relatedness and if the same is found in dolphins, this might represent a compelling case of convergent evolution.

Outline and summary of the data chapters

Chapter 2 – Ontogeny of male alliances

In Chapter two, published in *Behavioral Ecology* (Gerber et al., 2020), I adopted a long-term individual approach by investigating the ontogeny of male alliances in bottlenose dolphins. From our long-term database, I analysed data from 59 males while they transitioned from adolescence (8 to 14 years old) to adulthood (15 to 21 years old) and examined their genetic and social relationships in both age groups. For the first time, I could demonstrate that the vast majority of social bonds present in adolescence persisted into adulthood. This suggests that associations in early life foreshadow alliance membership. Pairwise relatedness predicted social bonds in adolescence but not adulthood. Rather than genetic similarity, age similarity predicted associations of adult males. The results of this chapter indicate that social bonds among peers influenced male alliance formation in bottlenose dolphins. Based on this finding, kinship seems to only play an ancillary role for male cooperation, suggesting that other evolutionary mechanisms than kin selection act on male cooperation in dolphins. However, based on my findings in Chapter 2 alone, I was not able to fully exclude kin selection as a mechanism, as kin availability during the formation of alliances was not considered.

Chapter 3 – Multi-level alliance partner choice

In Chapter 3 (submitted to Scientific Reports), I filled this important gap by taking partner availability into account, thereby addressing the question of whether kin availability (or the lack thereof) has a bearing on adult alliance formation. First, I identified second-order members of individual males as adults, as well as their preferred and avoided first-order alliance partners. Then, working backwards in time, I identified all males that retrospectively could have been potential second-order alliance partners of the individual male because of geographic and/or age overlap during their adolescence. Using this dataset, I investigated whether males preferably formed second-order alliances with kin, males of similar age and/or males they affiliated with most often and by proxy shared the strongest social bonds with. Supporting the findings of Chapter 2, I found no influence of relatedness on second-order alliance formation. Rather than kinship, social bond strength predicted second-order alliance member choice. Social bond strength was of particular importance when potential alliance members were younger, suggesting a preference for same-aged and older males. Similarly, first-order partner choice in consortships was influenced by social bond strength in the non-mating season. This is particularly interesting since first-order alliances consist of a small number of males and therefore, could be formed among relatives even when kin availability is low. By using an individual-based approach and accounting for partner availability, I was able to demonstrate that social bonds were

more important than kinship on both alliance levels. Kin selection, the most parsimonious explanation for male alliance formation, can thus be excluded, suggesting that other mechanisms providing direct fitness benefits have shaped the recent evolutionary history of male alliances in bottlenose dolphins.

Chapter 4 – The link between social bonds and reproductive success

Inspired by the findings of Chapters two and three which were suggestive of a high importance of social bonds on male cooperation within alliances, I explored if a male's social bonds to his second-order alliance members affected his reproductive success. I included various aspects of social relationships such as overall social bond strength to second-order alliance members (node strength) as well as variation in social bond strength and first-order alliance stability, *i.e.*, an individual's propensity to consort repeatedly with the same males. To assess the contribution of asocial variables on reproductive success, I further investigated the effects of age similarity and relatedness to second-order alliance members and individual home range size. I used the number of consortships as a proxy for reproductive success, after establishing via genetic paternity analyses that a male's number of consorthips and number of offspring sired were positively correlated. Node strength, as well as first-order alliance stability significantly predicted consortship success. My results highlighted that social bonds influenced fitness in allied males. Dolphins thus seem to be one of the few species where social attributes have a higher impact on male fitness compared to the traditionally investigated asocial variables.

My dissertation strongly suggests social bonds and not relatedness influence the formation and maintenance of alliances and thereby the reproductive success of male bottlenose dolphins. While it is impossible to conclude from my results whether male alliances in dolphins initially arose independently from kin selection or not, relatedness among allies appears to only play a negligible, if any, role. Combined, the chapters of this dissertation provide valuable insights into the underlying ultimate and proximate causes for male cooperation in bottlenose dolphins. Interestingly and similar as in humans, a male's social relationships to his alliance partners are more decisive characteristics of his fitness than his genealogical relationships and seem to do so throughout their lives.

“Make new friends, but keep the old; those are silver, these are gold.”

– Joseph Parry

“There's no friends like the old friends.”

– James Joyce

2. Affiliation History and Age Similarity Predict Alliance Formation in Adult Male Bottlenose Dolphins

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Abstract

Male alliances are an intriguing phenomenon in the context of reproduction since, in most taxa, males compete over an indivisible resource, female fertilisation. Adult male bottlenose dolphins (*Tursiops aduncus*) in Shark Bay, Western Australia, form long-term, multi-level alliances to sequester oestrus females. These alliances are therefore critical to male reproductive success. Yet, the long-term processes leading to the formation of such complex social bonds are still poorly understood. To identify the criteria by which male dolphins form social bonds with other males, we adopted a long-term approach by investigating the ontogeny of alliance formation. We followed the individual careers of 59 males for 14 years while they transitioned from adolescence (8-14 years of age) to adulthood (15-21 years old). Analysing their genetic relationships and social associations in both age groups, we found that the vast majority of social bonds present in adolescence persisted through time. Male associations in early life predicted alliance partners as adults. Kinship patterns explained associations during adolescence but not during adulthood. Instead, adult males associated with males of similar age. Our findings suggest that social bonds among peers, rather than kinship, play a central role in the development of adult male polyadic cooperation in dolphins.

Introduction

Social animals frequently interact with their group members. While such interactions can occur among different sets of individuals (e.g., Braun and Bugnyar, 2012; Henzi et al., 2009; Schweinfurth et al., 2017), some individuals interact repeatedly in beneficial contexts and over a longer period of time (Schino and Aureli, 2017). In many studies on wild animal populations, such long-term stable interactions among the same individuals have previously been referred to as 'social bonds' (Massen, 2017), defined as persistent, affiliative and cooperative relationships that may generate benefits through support which is approximately balanced between the partners (Ostner and Schülke, 2014; Silk, 2002). It has previously been shown that social bonds involve some degree of differentiation and selectivity in that individuals maintaining these bonds showed high rates of association (Ostner and Schülke, 2014; Silk, 2002; Thompson, 2019; Whitehead, 2008). Here, we follow this convention by referring to two individuals as having a social bond whenever they show significantly higher association rates than the group average over an extended period of time (Ostner and Schülke, 2014; Whitehead, 2008).

Benefits of social bonds include increased reproductive output (e.g., feral horses *Equus ferus*, Cameron et al., 2009; chimpanzees *Pan troglodytes*, Langergraber et al., 2013; Assamese macaques *Macaca*

assamensis, Schülke et al., 2010; house mice *Mus musculus*, Weidt et al., 2008), life span (e.g. savannah baboons *Papio cynocephalus*, Silk et al., 2010b), infant survival (e.g., savannah baboons, Silk et al., 2003; Silk et al., 2009), and decreased mortality (e.g., humans, Holt-Lunstad et al., 2010). Social bonds are thus crucial determinants of an individual's fitness. Fitting with predictions of kin selection theory (Hamilton, 1964), relatives are often preferred social partners, with closer kin being preferred over distant kin (reviewed in Smith, 2014).

Philopatry facilitates the formation of persistent social bonds and permits close association with kin post-weaning. Most mammals are characterised by female philopatry and male dispersal (Greenwood, 1980), allowing females to form close and enduring social bonds. This sex-bias can be explained by the different reproductive constraints faced by both sexes. While reproductive success of females is limited by access to resources and safety, male reproductive success is constrained by access to fertile females (Trivers, 1972).

Due to the indivisibility of fertilisations, a paradox exists where males cooperate to reproduce (Díaz-Muñoz et al., 2014). Males often engage in temporary coalitions, in which two or more males cooperate to compete against others in contests over resources or rank (Smith, 2014), thereby increasing individual access to females. Recurring coalitions among the same males, also known as male alliances (Harcourt, 1992), are an unusual phenomenon (Díaz-Muñoz et al., 2014). Alliances occur in, though are not limited to, humans (Chagnon, 1988), non-human primates (e.g., chimpanzees, Mitani et al., 2002a; savannah baboons, Noë, 1992), lions (*Panthera leo*, Packer et al., 1991), cheetahs (*Acinonyx jubatus*, Caro, 1994), lekking birds (e.g., manakins *Chiroxiphia linearis*, McDonald and Potts, 1994), fish (reviewed in Taborsky, 1994), and bottlenose dolphins (*Tursiops aduncus*, Connor et al., 1992).

Kin selection theory can resolve the apparent paradox of persisting social bonds between allied males when individuals are related (Caro, 1990; Pope, 1990; Qi et al., 2017). Polytocous species, those that give birth to multiple offspring at once, may produce 'ready-made' male alliances from single litters (Caro, 1990) or synchronised litters of related individuals (Packer et al., 1991). In contrast, many monotocous species, which produce single offspring, share the demographic challenge of maternal same-sex half-siblings being scarce and, if present, separated in age by at least several years. In chimpanzees, for instance, Langergraber et al. (2007) found that, although males do favour maternal siblings as allies, the majority of cooperative male dyads were formed among non-relatives.

In some species, for example long-tailed manakins and boat tailed grackles (*Quiscalus major*) (McDonald and Potts, 1994; Poston, 1997), non-kin male cooperation can be attributed to social

queuing, where subordinates remain in a group to acquire dominant status (Kokko and Johnstone, 1999). However, neither kin selection nor social queuing can explain the persisting social bonds formed among unrelated males observed in, for example humans (Boehm, 2009), baboons (Guinea baboons *Papio papio*, Patzelt et al., 2014), chimpanzees (Watts, 1998), Galapagos hawks (*Buteo galapagoensis*, Faaborg et al., 1995), and dolphins (Connor and Krützen, 2015; Wiszniewski et al., 2012b). To understand how persisting social bonds among unrelated males emerge, we need to investigate how, when and with whom such bonds are formed.

Male Indo-Pacific bottlenose dolphins in Shark Bay, Western Australia, exhibit one of the most remarkable male alliance systems known (Connor and Krützen, 2015). The system is unusual because male dolphins exhibit long-term, multi-level alliances with context-dependent interactions within an open social network (Connor et al., 2017; Connor et al., 2000b). This sets them apart from the multi-level alliances observed in baboon species (Grueter et al., 2012). While male dolphins in other populations form alliances (Owen et al., 2002; Parsons et al., 2003; Wiszniewski et al., 2012a), Shark Bay appears to be the only population where up to three levels of nested alliances occur (Connor and Krützen, 2015; Randić et al., 2012).

Alliance membership provides male dolphins with mating opportunities they would otherwise not obtain, as non-allied males have been shown to sire very few, if any, offspring (Krützen et al., 2004a). On the level of first-order alliances, two to three males cooperate to coerce individual oestrus females. Members of first-order alliances belong to larger groups of second-order alliances, consisting of 4-14 males, which compete against other such alliances over access to females (Connor et al., 1992; Connor et al., 2011). Males select their first-order allies from within their second-order alliance (Connor et al., 2011). Thus, second-order alliances are now recognised as the core social unit of male dolphins in Shark Bay (Connor and Krützen, 2015). Once formed in early adulthood, second-order alliances can persist over decades (Connor and Krützen, 2015). Adult male dolphins are characterised by high levels of association year-round (Smolker et al., 1992), increased rates of affiliative behaviours such as petting (Connor et al., 2006; Connor et al., 2000b) and high levels of cooperation during consortships (Connor and Krützen, 2015). Male alliances in dolphins therefore differ from the purely reproductive alliances or coalitions observed in other species (Díaz-Muñoz et al., 2014; Silk, 2002; Thompson, 2019). Male alliance partners in dolphins can therefore be said to exhibit social bonds based on the definition used in this study.

Preliminary work on relatedness among males in Shark Bay was ambiguous in that relatedness patterns differed markedly between alliances of different sizes and levels of stability (Krützen et al., 2003). However, Krützen et al. (2003) and studies on other dolphin populations (Diaz-Aguirre et al.,

2018; Möller et al., 2001; Parsons et al., 2003; Wiszniewski et al., 2012a) assessed the potential effect of kinship based on average group relatedness within established alliances relative to a population mean. In order to reveal the development of male alliance formation, however, individual males and their social bonds with others during alliance development need to be considered. Furthermore, the individual-based approach we employed here has an additional advantage in that it accounts for the availability of an individual's potential associates, unlike earlier studies assuming equal availability of potential associates.

Previous work demonstrated that male calves and juveniles (two years pre- and two years post-weaning, respectively) invest more time into developing social skills compared to females of the same age (Krzyszczyk et al., 2017; Stanton et al., 2011). The persistence of specific dyadic associations, though, has never been tested. Adolescence is a critical period during which males become sexually and socially mature (Connor and Krützen, 2015). In contrast to adult males, adolescent males have not yet established alliance membership and do not engage in consortships. Thus, patterns of associations are expected to differ qualitatively and quantitatively between adolescent and adult males. To close this important gap in our understanding of alliance formation, we explored the development and maintenance of social bonds during the transition from adolescence to adulthood.

Here, we describe the ontogeny of social bonds and thus, alliance formation among male bottlenose dolphins relative to patterns of genetic relatedness, affiliation history, and age-similarity. We focus on social bonds of individual males during the transition from adolescence to adulthood. This approach permitted us to gain insights into the underlying dynamics of friendships among male bottlenose dolphins.

Materials and Methods

Data collection and study subjects

Data were collected at our long-term field site in the eastern gulf of Shark Bay, Western Australia. Standardised observations, hereafter 'surveys', have been conducted on this Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) population since 1984 (Connor and Krützen, 2015). In this study, we extracted survey data on group composition recorded between the years 1988 and 2015. Group composition was determined during the first five minutes of each survey according to the 10-m 'chain rule' using photographs of dorsal fins to identify individuals (Smolker et al., 1992).

We extracted association data on 59 focal males, each of which we followed over 14 years. Data were extracted for two periods in the lifespan of each focal male, creating two age groups: from eight to 14 years ('adolescence'), and from 15 to 21 years old ('adulthood'). By age eight, male bottlenose dolphins have typically been weaned for approximately four years and have transitioned to being independent juveniles (Mann et al., 2000a). Male bottlenose dolphins reach sexual maturity between twelve and 15 years of age (Kemper et al., 2014) and start forming stable alliances at approximately 15 years of age (Connor et al., 2000b). Only alliance membership enables males to successfully consort females (Connor et al., 1992). Males under 13 years of age have never been observed consorting an adult female, although this does not exclude the possibility that some males might start at a younger age (Connor and Krützen, 2015). To avoid biases introduced by unbalanced age ranges, we limited the upper age in the adult category to 21 years of age, although dolphins may live past 40 in this population (unpublished data).

Whenever possible, birth dates were assigned based on the first sighting of a dolphin as a calf. Calf age was estimated using behavioural and physical criteria, including surfacing patterns, presence of foetal folds, as well as the last sighting of the mother before birth (Smuts and Mann, 1999). In cases where there were no early-life demographic data available, we used ventral speckle density to infer approximate birth date, which is highly correlated with age in our study population (Krzyszczyk and Mann, 2012).

Association rates

Socio-positive behaviours such as grooming in primates (Dunbar, 2010) are often used to infer social bond strength on the qualitative level. However, social bonds can also be identified quantitatively by increased rates of affiliation over a prolonged period of time compared to other relationships in the

group (Ostner and Schülke, 2014; Silk, 2002; Thompson, 2019; Whitehead, 2008). Since socio-positive behaviours cannot be observed systematically underwater, we used association rates to identify social bonds among male dolphins. Association rates are well-established indicators for social bond strength in cetaceans (Mann et al., 2000b) and have been demonstrated to correlate with social bond strength (Mitani, 2009; Silk, 2002). To quantify associations between focal males and their male associates, we calculated Half-Weight Association Indices (HWIs, Cairns and Schwager, 1987) for each focal male twice: once during its adolescence and for a second time while adult. A focal male's associates consisted of all males with whom a focal was observed. Both focal males and all their associates had to have been observed at least 30 times in each age group. We used *SOCPROG* v2.6 (Whitehead, 2009) to generate HWIs with the sampling period set to one day. Group composition data was collected during surveys, and re-sights, *i.e.,* same group encountered within two hours, were excluded.

To identify social bonds among focal males and their associates in each age group, we conducted a change point analysis. Change points are those where statistical properties of the data to either side differ, as is expected for different levels of social structures in multi-level societies (e.g., Patzelt et al., 2014; Wittemyer et al., 2005). To locate change points, we used the *changepoint* (Killick and Eckley, 2014) R package employing the Pruned Exact Linear Time (PELT) method. The dataset for the change point analysis comprised all dyads associating during the focal male's adulthood, denoted by a HWI larger than 0 (N = 1,755). Adulthood HWIs were higher (mean ± sd = 0.12 ± 0.18, N = 1,755) than those in adolescence (mean ± sd = 0.10 ± 0.13, N = 1,544). Including adulthood HWIs only resulted in a more conservative cut-off for the adolescence period while reliably identifying a focal male's alliance partner once adulthood was reached.

Based on the changepoint analysis, we defined a cut-off HWI value above which we regarded two males as sharing a social bond due to association rates well-above random expectations (Ostner and Schülke, 2014; Silk, 2002; Whitehead, 2008). For focal adolescent males and any other male above the cut-off value, we chose the term 'close associates'. Correspondingly, for focal adult males and any other male above the cut-off, we used 'alliance partners'. Exploring the dyadic nature of male bonds in two separate age periods allowed us to determine some of the proximate mechanisms that predict alliance formation in adult male dolphins for the first time.

Pairwise relatedness and mitochondrial matriline membership

To explore the potential role of kinship in male association patterns, we used both maternal mitochondrial DNA (mtDNA) haplotypes and pairwise autosomal relatedness estimates. DNA was extracted from tissue samples which were collected using a remote biopsy system for small cetaceans

(Krützen et al., 2002). To assign individuals to their maternal haplotype, we sequenced a 468 base-pair fragment of the hypervariable region I of mtDNA, as described in Bacher et al. (2010). We estimated pairwise relatedness based on 22 hypervariable autosomal microsatellite markers, which we generated and scored using previously established protocols (Kopps et al., 2014a). We provide summary statistics on genetic diversity indices in the supporting information.

Relatedness estimators perform differently based on the inherent relatedness composition of a population (Csillery et al., 2006). We identified the best performing relatedness estimator (*i.e.*, high precision, low standard deviation) for our population by choosing the estimator with the highest correlation between simulated relatedness estimates and true relatedness values, *i.e.*, the values expected for a certain relationship, using the software *Coancestry* v1.0.1.5 (Wang, 2011). We included all individuals with no more than three missing loci and simulated 1,000 relatedness values based on the allele frequency distribution in our population. Simulations were completed for each of the following relationships: parent-offspring (PO), half-siblings (HS), and unrelated individuals (UR). We then compared the correlations between the simulated values and the expected true relatedness values (PO = 0.5, HS = 0.25, UR = 0) for all estimators. The triadic likelihood estimator TrioML (Wang, 2007) showed the highest correlation and was therefore our relatedness estimator of choice.

Male-male associations during adolescence and adulthood

We quantified the effect of shared mtDNA haplotype, pairwise relatedness, age, and association rates during adolescence on adulthood association rates by building two linear mixed models. For both models, the response variables were the HWIs between focal males and their close associates during adolescence, and their alliance partners in adulthood, respectively. Advancing upon previous research in this area (Diaz-Aguirre et al., 2018; Krützen et al., 2003; Möller et al., 2001; Parsons et al., 2003; Wiszniewski et al., 2012a), this approach allowed us to analyse the ontogeny of alliance formation on an individual level during this critical period.

For the adolescence model (Model 1), the explanatory variables we tested were: age difference in years, pairwise relatedness, and the presence or absence of a shared mtDNA haplotype. In the adulthood model (Model 2), we investigated association patterns of the focal males during their adulthood and included the same variables as Model 1, as well as a dyad's HWI during the focal male's adolescence. This additional variable permitted us to test whether a dyad's association during the focal male's adolescence predicts their association once the focal male reached adulthood. To control for repeated measures of individuals, we included the ID code of the focal male and his close

associates (Model 1) or alliance partners (Model 2) as random effects. All analyses were carried out in R v3.4.0 and linear mixed models were built using the R package *lme4* (Bates et al., 2015).

To identify which combination of the above variables best described the observed association patterns during adolescence and adulthood, we first calculated a global model including all variables (Table S2.1: Models 1 and 2). Subsequently, we used the 'dredge' function in *MuMIn* (Barton and Barton, 2018) to obtain second-order Akaike's Information Criterion values corrected for small sample size (AICcs) for models containing only a subset of variables. Differences in AICc (ΔAICc) allowed us to determine the best models among candidate solutions (Zuur et al., 2007). We then averaged across the top model set separately for the adolescence (Model 1) and the adulthood model (Model 2), where ΔAICc < 2 (Burnham and Anderson, 2002; Grueber et al., 2011). All models with ΔAICc < 2 are shown in Table 2.1.

Table 2.1. Models with ΔAICc < 2 compared to the top models.

	Model	*AICc*	*Δ AICc*	*Weight*
1a	$HWI_{adolescence}$ ~ r + Δage + (1|ID_F) + (1|ID_A)	-304.72	0	0.42
1b	$HWI_{adolescence}$ ~ r + (1|ID_F) + (1|ID_A)	-304.54	0.18	0.39
1c	$HWI_{adolescence}$ ~ r + Δage + haplotype + (1|ID_F) + (1|ID_A)	-303.11	1.6	0.19
2a	$HWI_{adulthood}$ ~ $HWI_{adolescence}$ + Δage + (1|ID_F) + (1|ID_A)	-448.1	0	0.63
2b	$HWI_{adulthood}$ ~ $HWI_{adolescence}$ + haplotype + Δage + (1|ID_F) + (1|ID_A)	-446.99	1.1	0.37

Results of model selection including model details of all models with ΔAICc < 2. Models 1a-c were averaged to identify the effect of relatedness (r), shared haplotype, and age difference (Δage) on associations during adolescence. Individual ID codes are included as random effects (ID_F = ID focal, ID_A = ID Close Associate). Models 2a-b were averaged to explore how associations during adolescence, age difference, and shared haplotype influence adulthood association patterns. For the adulthood models, ID_A denotes alliance partners but all other abbreviations are consistent with the ones described for models 1a-c. Relatedness was not included in the best adult models. Weight describes the proportion of a given model to the averaged model.

Within-dyad relationship development

We wanted to determine whether age, relatedness, or shared mtDNA haplotype influenced the formation, maintenance or termination of social bonds between adolescence and adulthood. For this, we combined data from the previous analyses to classify each dyad in one of the following three categories of within-dyad relationship development ('*RD'*): close associates during adolescence and alliance partners in adulthood ('*ASA'*); close associates during adolescence but not alliance partners in adulthood ('*ASO'*); alliance partners in adulthood without having been close associates during adolescence ('*AO'*).

We built a total of nine binomial generalised linear mixed models (Table S2.1: Models 4 a-c), in which we entered either pairwise relatedness, shared mtDNA haplotype or age difference as explanatory variables and investigated their effect on the three relationship development scenarios. The variables were tested separately because models including combinations of the explanatory variables did not converge. To account for multiple tests, we adjusted the significance level to 0.0056 (Bonferroni correction, Dunnett, 1955). In models (a), we were interested in whether males who became alliance partners without having been close associates (AO) differed in either age, relatedness or shared haplotype from males that became alliance partners and were close associates during adolescence (ASA). In models (b), we investigated if any of the three variables were correlated with whether close associates during adolescence became alliance partners once adulthood was reached (ASA) or not (ASO). Lastly, in models (c), we tested whether male dyads with social bonds only during either adolescence or adulthood *i.e.,* either being close associates (ASO) or alliance partners (AO) only, differed with respect to age difference, relatedness or haplotype similarity.

Statistical significance of fixed effects was assessed using Satterthwaite's method as implemented in the package *lmertest* (Kuznetsova et al., 2015). All models presented above were validated by likelihood ratio tests where we compared each model to a null model containing only the intercept and random effects. All models performed significantly better than their respective null models. To test for multicollinearity among our explanatory variables, we calculated the Variance Inflation Factors (VIFs), running the script containing the 'corvif' function provided by Zuur (2009). None of our variables were correlated (Table S2.3). Lastly, we checked the model assumptions visually by Q-Q plots, as well as histograms of the residuals (provided in the supporting information). Interactions were not significant in all models and were, thus, not incorporated.

Results

Identification of 'close associates' and 'alliance partners'

We identified three change points (Fig. 2.1) reflecting the multi-level social structure of male associations in our population. The first change point, observed at HWI of ≥ 0.38, separates first-order alliance partners from second-order alliance partners (N = 201, mean = 0.55). The second change point, at HWI ≥ 0.19, divides second-order alliance partners from males that occasionally associate (N = 160, mean = 0.28). The third change point, at HWI ≥ 0.09, separates males that occasionally associate (N = 182, mean = 0.12) from those that randomly associate (N = 1,212, mean = 0.03). We treated all dyads with a HWI ≥ 0.19 (*i.e.,* the second change point detected) as close associates during adolescence. The same value was used to identify alliance partners once the focal males transitioned into adulthood. This value is supported by earlier studies on the same population, showing that a HWI ≥ 0.2 validly clustered males into their respective second-order alliances (Connor et al., 2001; King et al., 2018; Smolker et al., 1992). Furthermore, the identified value of HWI ≥ 0.19 is well above average rates of association (mean HWI = 0.12 ± 0.17, excluding zeros, N = 1,755). Since HWIs were calculated per age period, they reflect increased levels of association over a prolonged period of time. Thus, this value indicates differentiated relationships among male dolphins that fit our definition of social bonds.

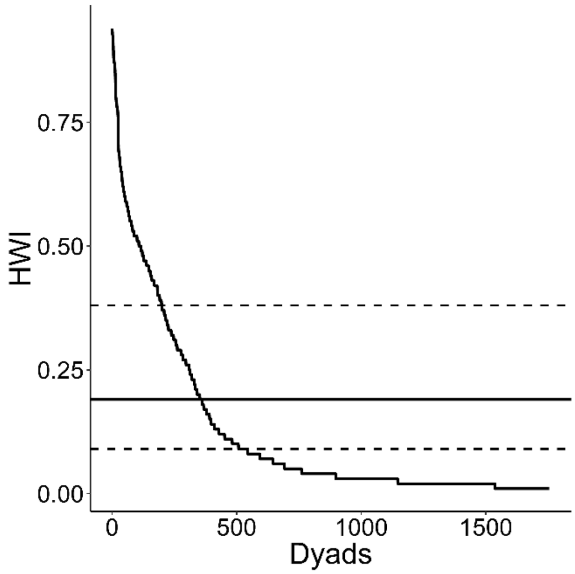

Figure 2.1. Change point analysis showing HWI values defining various levels of association. Distribution of adulthood HWI among 1,755 dyads of focal males and all their male associates, with lines representing change points. The continuous line denotes the HWI value of 0.19 separating males with social bonds above the line from males that associate occasionally below. The dashed line (HWI = 0.38) differentiates second-order from

first-order alliance partners. The dotted line (HWI = 0.09) separates random associations from occasional associates.

Male-male associations during adolescence and adulthood

During adolescence, pairwise relatedness significantly correlated with HWI (z = 2.974, *P* = 0.003, Table 2.2), indicating that adolescent focal males associated more frequently with related males. Age difference between a focal adolescent male and its close associates, as well as shared mtDNA haplotype, were not significant predictors of association patterns during adolescence.

Table 2.2. Results of averaged models on male association rates during adolescence and adulthood.

	β		*S.E.*		*z-value*		*P-value*	
	Adolescent	Adult	Adolescent	Adult	Adolescent	Adult	Adolescent	Adult
Intercept	0.352	0.426	0.014	0.015	26.016	29.053	**< 0.001**	**< 0.001**
Relatedness	0.047	NA	0.016	NA	2.947	NA	**0.003**	NA
Haplotype	0.002	0.005	0.009	0.011	0.269	.474	0.788	0.636
$HWI_{Adolescent}$	NA	0.187	NA	0.016	NA	11.844	NA	**< 0.001**
Age difference	-0.019	-0.039	0.020	0.017	0.927	2.314	0.354	**0.0207**

Results of averaged Models 1a-c and averaged Models 2a-b (Table 2.1), investigating the effect of age similarity, HWI during the focal male's adolescence (Models 2), pairwise relatedness and shared haplotypes on association patterns during adolescence and adulthood in males.

Once focal males reached adulthood, the averaged model indicated age similarity (z = 2.314, *P* = 0.021, Table 2) and adolescence HWI (z = 11.844, *P* < 0.001, Table 2.2) as significant predictors explaining patterns of association. These results suggested that adult males affiliated more often with males of similar age, as well as males with whom they already closely associated during adolescence (Fig. 2.2). In contrast to the best adolescence models, pairwise relatedness was not included in the best adulthood models and, thus, did not hold explanatory power for male associations. As in the adolescence model, shared mtDNA haplotype was not significant.

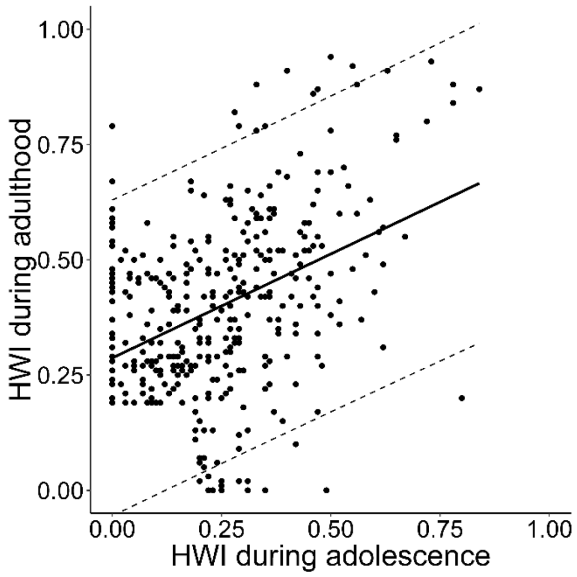

Figure 2.2. Positive correlation between adolescent and adult association rates within dyads. The association rate between an adolescent male and a second individual predicts the same dyad's association rate once a male matured into an adult, suggesting adolescent close associates foreshadow alliance partners (N = 361 dyads, Model 2). The dashed lines enclose the 95% confidence interval.

Within-dyad relationship development

We identified 209 dyads (53%) that were close associates during adolescence and became alliance partners in adulthood (ASA). In contrast, relatively few dyads (N = 32, 8%) were close associates during adolescence without becoming alliance partners in adulthood (ASO). Notably, this was not due to some individuals missing from the adulthood dataset because of permanent emigration or death; instead, this result may reflect selective termination of social bonds. We found 152 dyads (39%) that became alliance partners during adulthood without having closely associated during adolescence (AO). This result implies a significant overall net gain of social bonds during the transition from adolescence to adulthood (mean close associates ± se adolescent males = 4.1 ± 3.2; mean alliance partners adulthood ± se = 6.1 ± 3.8; Poisson Generalised Linear Mixed Model, z = 4.414, se = 0.083, $P < 0.001$; Fig. 2.3, Table S2.1: Model 3). To ensure that this result was not biased by the conservative cut-off of HWI ≥ 0.19 for adolescence, we re-ran the change point analysis including adolescence HWIs > 0 only. The change point separating close associates during adolescence was found at HWI ≥ 0.15. On average, individual males still had significantly more social bonds in their adulthood (mean close associates ± se adolescence males = 4.9 ± 3.4; mean alliance partners ± se adulthood = 6.1 ± 3.8; Poisson Generalised Linear Mixed Model, z = 2.728, se = 0.078, $P = 0.006$).

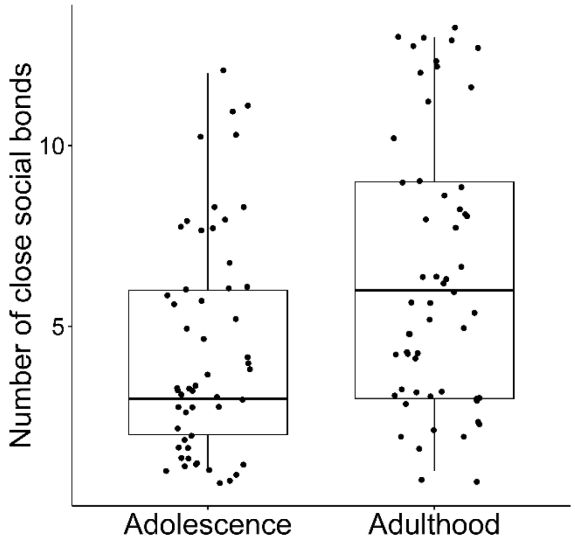

Figure 2.3. Increase in number of social bonds during the transition from adolescence to adulthood. Boxplot showing the average number of social bonds during the focal male's adolescence (*i.e.,* number of close associates) and adulthood (*i.e.,* number of alliance partners) including 95% confidence intervals. The average number of social bonds (HWI ≥ 0.19) is higher in a male's adult age bracket compared to its adolescence. This result suggests that males form additional social bonds during the transition into adulthood (Poisson Generalised Linear Mixed Model, including age period, *i.e.,* adolescence or adulthood, as explanatory, number of close associates/alliance partners as dependent, and IDs as random variable, $P < 0.001$, N = 118, Model 3).

The within-dyad relationship models (Models 4) showed no effect of either shared haplotype or pairwise relatedness on within-dyad relationship development between adolescence and adulthood (Table 2.3, *Hap., r*). Hence, neither the persistence nor the formation of social bonds between adolescence and adulthood seemed to be influenced by relatedness. However, age difference was significant in two out of the three models (Table 2.3, Δage). Dyads that were close associates in adolescence but did not become alliance partners in adulthood (ASO) had a significantly larger age difference (mean ± sd = 8.6 ± 7.1 years) than dyads that were close associates during adolescence and became alliance partners later on (ASA, mean age difference 3.5 ± 3.4 years; Binomial Generalised Linear Mixed Model, $P < 0.001$). These results indicated that similarly aged males were more likely to form persistent social bonds and, thus, become alliance partners. We also found significantly larger age differences between males who were close associates during adolescence without becoming alliance partners (ASO, Binomial Generalised Linear Mixed Model, $P < 0.001$) compared to dyads that were alliance partners but had not been close associates (AO, mean age difference 3.8 ± 3.1 years). It

appeared that adult males formed social bonds with other males of more similar age than they did during adolescence.

Table 2.3. Results from within-dyad relationship analyses.

	B			*S.E.*			*z-value.*			*P-value*		
	r	Δage	Hap.	r	Δage	Hap.	r	Δage	Hap.	r	Δage	Hap.
ASA-AO	1.54	-0.10	0.36	1.06	0.05	0.20	1.45	-2.04	1.86	0.15	0.04	0.06
ASA-ASO	-0.11	-5.45	-0.67	5.89	1.11	1.50	-0.02	-4.90	-0.45	0.99	**<0.001**	0.66
ASO-AO	-20.08	1.38	3.37	11.80	0.24	2.13	-1.70	5.70	1.59	0.089	**<0.001**	0.11

Results of Models 4 a-c), top to bottom. ASA = close associates during adolescence and alliance partners in adulthood, ASO = close associates during adolescence without becoming alliance partners in adulthood, and AO = alliance partners in adulthood but not close associates during adolescence. r = pairwise relatedness, Δage = age difference in years, and Hap. = shared or non-shared haplotype. *P*-values in bold indicate significant results after Bonferroni correction.

Discussion

Affiliation history and age similarity predicted social bonds and thus, alliance partnerships among adult male bottlenose dolphins. Individual social bonds formed during adolescence, particularly those among similar-aged males, persisted into adulthood. Kinship explained social bonds of adolescents, most likely due to bisexual philopatry (Krützen et al., 2004b; Tsai and Mann, 2013). However, kinship did not predict the persistence of social bonds into adulthood, or the existence of social bonds among adult males. Social bonds were maintained during the transition from adolescence to adulthood, yet, relatedness no longer predicted patterns of associations among adult dolphins. This effect is likely due to males increasing their number of social bonds between adolescence and adulthood.

Our finding that association rates during adolescence were positively correlated with relatedness may be best explained by associations with their mothers' related associates. In Shark Bay, adult females maintain high association rates with female relatives (Frère et al., 2010b). This is in line with recent work that social networks in a large range of species, including bottlenose dolphins (Ilany and Akçay, 2016), were shaped by social inheritance of maternal bonds (Goldenberg et al., 2016; Kerhoas et al., 2016).

Simple rules based on proximity and familiarity, without requiring sophisticated mechanisms to distinguish kin from non-kin, may operate where partner choice is kin biased. This is found in many primate species characterised by female philopatry (e.g., cercopithecine primates, Chapais, 2002). Persistent social bonds during the transition from adolescence to adulthood suggest that male dolphins might apply the same rules. Yet, the outcome if very different when individuals are expected to have access to a limited number of close relatives and where kin discrimination may be limited (Lewis et al., 2013a). Moreover, in order to maximise individual fitness, the identification of competent and compatible alliance partners may be more important than kin biased partner choice. Traits that might be valued in alliance partners include resource holding potential (Parker, 1974), cooperativeness (Heinsohn and Packer, 1995), including the propensity to return received favours (Taborsky et al., 2016), competence (Chapais, 2006), and other characteristics, such as behavioural homophily (e.g., in chimpanzees, Massen and Koski, 2014) or dominance rank (e.g., hyenas *Crocuta crocuta*, Smith et al., 2007). The amount of time that individuals have been associated and their relationship quality may also be important factors influencing partner choice, as recently shown in female mangabeys (*Cercocebus atys*) and chimpanzees (Mielke et al., 2018), as well as ravens (*Corvus corax*, Asakawa-Haas et al., 2016). Furthermore, a modelling approach as well as experimental evidence from humans suggests that individuals are more likely to cooperate with previously helpful

partners (Campennì and Schino, 2014; Wang et al., 2012). Multiple positive experiences with the same partners can thus lead to persisting social bonds, although this may not always be the case (Schweinfurth and Taborsky, 2018).

Adult male bottlenose dolphins in Shark Bay primarily cooperate to sequester oestrus females within consortships, or in conflicts over access to females with other alliances; the latter entailing risk of injury (Connor and Krützen, 2015; Connor et al., 2011; Hamilton et al., 2019). Hence, a male's reproductive success is not only determined by his own competence but also by that of his alliance partners, and how well these individuals can synchronise and coordinate their joint behaviours (Connor et al., 2006). Evidence that males selectively choose partners for activities whose payoff is affected by partner competency, independently of kinship, has also been described in chimpanzees. Males will participate in alliances, take part in risky boundary patrols, or share meat with the males that they either associate with most often and trust to take the same risk (Watts and Mitani, 2001), or with males of similar age and rank, rather than kin (Mitani et al., 2000; Mitani et al., 2002b).

Bisexual philopatry and a slow life history, including a long developmental period, provide male dolphins in Shark Bay with opportunities to assess each other's competencies and compatibilities, as well as to form social bonds with desirable future alliance partners. Indeed, similar to chimpanzees (Kawanaka, 1989; Pusey, 1990), rats (*Rattus norvegicus*, Auger and Olesen, 2009), and elephants (*Loxodonta africana*, Evans and Harris, 2008), juvenile male dolphins in Shark Bay invest more time into social activities compared to females (Krzyszczyk et al., 2017). Social activities often involve play-herding, a behaviour where adolescents practice consortship behaviour (Connor et al., 2000b), which potentially serves to increase efficacy of future consortships through joint practice or to identify valuable alliance partners.

The persistence of social bonds during the transition from adolescence to adulthood implies that the actual formation of alliances is a protracted process, likely starting years before males become reproductively active. Even though social bonds among unrelated, similar-aged males could also arise by demographic constraints without invoking partner choice (Connor et al., 2000a), our finding of persistent social bonds and the fact that they are subject to directional changes suggest otherwise. During the transition from adolescence to adulthood, focal males increased the overall number of social bonds with new social bonds typically arising among male dolphins of similar age. The few social bonds that were discontinued were observed among males with larger age disparities. Hence, male dolphins showed a preference for a larger number of similar-aged alliance partners.

In bats, individuals can benefit from expanding their social networks by forming social bonds with non-kin (*Desmodus rotundus*, Carter et al., 2017). Individuals with larger networks consisting of kin and non-kin alike coped better with partner loss compared to individuals who had smaller networks consisting predominantly of kin. Considering the importance of alliance partners for male dolphins, expanding the social network with valuable non-kin individuals might lead to an increase in reproductive success and, thus, fitness. Under such a scenario, males might benefit from a larger number of alliance partners composed of kin and non-kin alike. Because of the different constraints on reproductive success between the sexes, males might prefer a large number of competent but unrelated partners. This is not necessarily the case for females. Therefore, social bond formation might underlie different criteria in the two sexes and might explain why males form social bonds with non-kin, whereas the social partners of females usually consist of kin (Frère et al., 2010b; Möller et al., 2006).

Male bottlenose dolphins might apply similar rules based on proximity, homophily and familiarity for alliance formation as other species. Due to their social system however, these do not necessarily lead to kin-biased cooperation. Thus, rather than kin selection, we propose that other evolutionary mechanisms, such as forms of intra-species mutualism (Connor, 1986), reciprocity (reviewed in Taborsky et al., 2016; Trivers, 1971a) or a combination thereof, should be invoked when explaining the evolution of social bonds in male bottlenose dolphins (reviewed in Connor, 2010). Our results reveal that long-term familiarity and age similarity, but not necessarily relatedness, influence the formation of multi-level alliances in male Indo-Pacific bottlenose dolphins. With this study, we add to the growing body of research highlighting the occurrence of enduring social bonds in animals, independent of relatedness among friends.

Acknowledgements

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Supporting Information

Table S2.1. Overview of all (generalised) linear mixed models.

	Sample size	*Model*
Global Model 1: Association patterns during adolescence	- 241 dyads - 54 focal males* - 82 close associates	$HWI_{adolescence} \sim r + haplotype + \Delta age + (1|ID_F) + (1|ID_A)$
Global model 2: Association patterns during adulthood	- 361 dyads - 57 focal males* - 85 alliance partners	$HWI_{adulthood} \sim HWI_{adolescence} + r + haplotype + \Delta age + (1|ID_F) + (1|ID_A)$
Model 3: Number of close associates vs. number of alliance partners	- 59 focal males	$\#CA_F \sim age\ period + (1|ID_F)$
Model 4: Within-dyad relationship development	a) Same as Model 2 b) Same as Model 1 c) 53 focal males & 71 close associates	a) AO vs ASA ~ Δage *or* r *or* haplotype + $(1|ID_F) + (1|ID_A)$ b) ASO vs ASA ~ Δage/r/haplotype + $(1|ID_F) + (1|ID_A)$ c) ASO vs AO ~ Δage *or* r *or* haplotype + $(1|ID_F) + (1|ID_A)$

Overview of all (generalised) linear mixed models describing sample sizes and variables investigated (r = pairwise relatedness; Δage = age difference in years, $\#CA_F$ = total number of a focal male's close associates during adolescence and number of alliance partners during adulthood, RD = within-dyad relationship development, ID_F = ID focal, ID_A = ID Close Associate / Alliance Partner). ASA = close associates during adolescence and alliance partners in adulthood, ASO = close associates during adolescence not becoming alliance partners in adulthood, and AO = alliance partners during adulthood but not close associates during adolescence. Most individuals were close associates or alliance partners of more than one focal male. Hence, the number of dyads is higher than the sum of focal males and close associates / alliance partners. *Five individuals had no close associates as adolescents, whereas two had no alliance partners in their adult age bracket.

Summary statistics of microsatellite markers

Our study area encompasses a larger area than the individual dolphins' home ranges (Randić et al., 2012). To test for underlying substructure as reported in a previous publication (Krützen et al., 2004b) we ran a STRUCTURE analysis (Pritchard et al., 2000) with the default parameters on our loci. The most likely number of clusters was K = 3 (Fig. S2.2), hereafter referred to as Pop1, Pop2, and Pop3. All summary statistics were calculated in GenAlex v6.5 (Peakall and Smouse, 2012) and are reported per cluster.

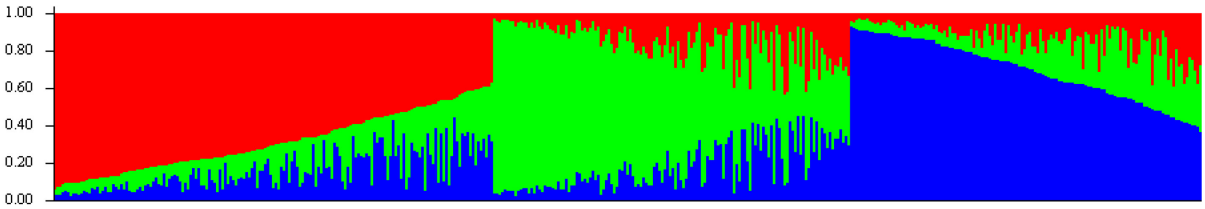


Figure S2.1. Population substructure of Shark Bay dolphins. Bar plot of K = 3 (top) showing population substructure in Shark Bay.

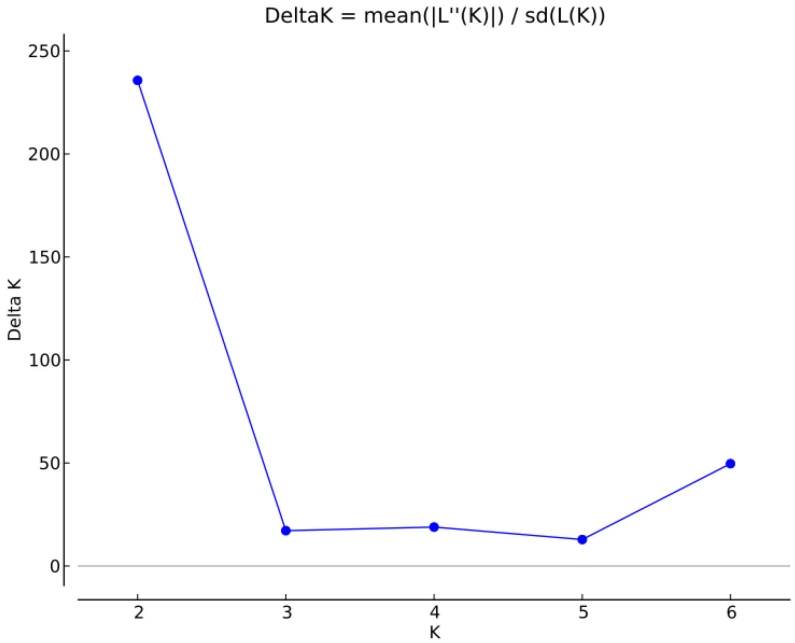


Figure S2.2. Delta K plot to identify most likely number of clusters. Plot of number of clusters (K) and the respective change of K (Delta K) to the previous cluster. The largest Delta K is observed between two and three clusters, suggesting population substructure of three clusters in Shark Bay. Figure created with STRUCTURE HARVESTER (Earl and von Holdt, 2012).

Table S2.2. Summary Statistics on microsatellite markers.

	Locus	N	Na	Ne	I	Ho	He	uHe	F
Pop1	E12	121	5	2.861	1.206	0.661	0.651	0.653	-0.016
	MK6	121	12	7.796	2.226	0.893	0.872	0.875	-0.024
	T105	118	5	3.150	1.247	0.661	0.682	0.685	0.031
	T108	120	2	1.536	0.533	0.333	0.349	0.350	0.044
	T111	121	5	1.440	0.663	0.331	0.306	0.307	-0.081
	T117	121	5	2.758	1.145	0.628	0.637	0.640	0.015
	T128	120	5	3.178	1.301	0.583	0.685	0.688	0.149
	T66	119	5	1.206	0.378	0.168	0.171	0.172	0.017
	T98	121	2	1.820	0.643	0.455	0.451	0.453	-0.009
	D22	118	5	2.989	1.297	0.669	0.665	0.668	-0.006
	D8	117	3	2.262	0.947	0.547	0.558	0.560	0.020
	F10	116	4	2.745	1.156	0.612	0.636	0.638	0.037
	T138	121	7	2.275	1.164	0.455	0.560	0.563	0.189
	T141	121	10	5.848	1.942	0.785	0.829	0.832	0.053
	T87	121	3	1.706	0.733	0.372	0.414	0.415	0.101
	T91	121	8	4.211	1.710	0.777	0.763	0.766	-0.019
	T162	120	3	1.938	0.751	0.442	0.484	0.486	0.087
	MK9	120	5	4.323	1.512	0.733	0.769	0.772	0.046
	MK5	121	5	4.294	1.510	0.802	0.767	0.770	-0.045
	T132	121	3	1.499	0.587	0.355	0.333	0.334	-0.067
	KWM12	120	9	5.038	1.815	0.817	0.802	0.805	-0.019
	EV37	107	12	3.913	1.804	0.645	0.744	0.748	0.134
	T80	119	9	3.679	1.526	0.782	0.728	0.731	-0.073
	MK3	120	8	3.228	1.435	0.700	0.690	0.693	-0.014
	T142	121	4	1.762	0.793	0.413	0.433	0.434	0.045
	T153	121	2	1.308	0.398	0.256	0.236	0.237	-0.088
	MK8	118	9	2.703	1.279	0.678	0.630	0.633	-0.076
Pop2	E12	87	4	2.961	1.229	0.667	0.662	0.666	-0.007
	MK6	87	14	5.563	2.060	0.782	0.820	0.825	0.047
	T105	87	7	2.957	1.333	0.701	0.662	0.666	-0.059
	T108	87	2	1.950	0.680	0.494	0.487	0.490	-0.015
	T111	86	5	1.729	0.850	0.430	0.422	0.424	-0.021
	T117	87	3	2.182	0.890	0.540	0.542	0.545	0.003
	T128	84	5	2.483	1.125	0.595	0.597	0.601	0.003
	T66	87	4	1.177	0.333	0.149	0.150	0.151	0.006
	T98	87	2	1.775	0.628	0.437	0.437	0.439	-0.001
	D22	87	5	2.350	1.122	0.575	0.574	0.578	0
	D8	86	3	2.197	0.915	0.581	0.545	0.548	-0.067
	F10	86	4	2.465	1.045	0.709	0.594	0.598	-0.193
	T138	87	5	2.184	1.064	0.563	0.542	0.545	-0.039
	T141	87	10	4.692	1.813	0.793	0.787	0.791	-0.008
	T87	87	5	1.727	0.813	0.402	0.421	0.423	0.045
	T91	87	8	4.401	1.679	0.885	0.773	0.777	-0.145
	T162	87	3	2.046	0.797	0.471	0.511	0.514	0.078

	MK9	87	4	3.465	1.293	0.724	0.711	0.716	-0.018
	MK5	87	6	4.210	1.503	0.770	0.762	0.767	-0.010
	T132	87	2	1.047	0.109	0.046	0.045	0.045	-0.024
	KWM12	87	10	5.918	1.930	0.839	0.831	0.836	-0.010
	EV37	82	9	5.220	1.870	0.817	0.808	0.813	-0.011
	T80	87	9	4.722	1.728	0.770	0.788	0.793	0.023
	MK3	86	7	2.737	1.369	0.640	0.635	0.638	-0.008
	T142	87	4	1.776	0.841	0.414	0.437	0.440	0.053
	T153	87	2	1.548	0.539	0.299	0.354	0.356	0.156
	MK8	87	6	3.856	1.497	0.805	0.741	0.745	-0.086
Pop3	E12	112	6	3.305	1.319	0.723	0.697	0.701	-0.037
	MK6	112	15	8.708	2.345	0.875	0.885	0.889	0.011
	T105	111	6	2.807	1.215	0.658	0.644	0.647	-0.022
	T108	112	2	1.733	0.614	0.339	0.423	0.425	0.198
	T111	112	4	1.583	0.664	0.286	0.368	0.370	0.224
	T117	112	5	1.999	0.914	0.536	0.500	0.502	-0.072
	T128	108	4	1.859	0.876	0.444	0.462	0.464	0.038
	T66	112	5	1.645	0.815	0.384	0.392	0.394	0.021
	T98	112	2	1.898	0.666	0.482	0.473	0.475	-0.019
	D22	108	5	2.017	0.965	0.546	0.504	0.507	-0.083
	D8	110	3	2.361	0.965	0.591	0.576	0.579	-0.025
	F10	111	5	2.444	1.111	0.622	0.591	0.594	-0.052
	T138	112	5	3.289	1.316	0.607	0.696	0.699	0.128
	T141	112	11	6.635	2.062	0.848	0.849	0.853	0.001
	T87	112	5	1.557	0.733	0.384	0.358	0.360	-0.073
	T91	112	8	3.817	1.570	0.813	0.738	0.741	-0.101
	T162	112	3	1.591	0.684	0.268	0.371	0.373	0.279
	MK9	112	5	3.401	1.336	0.705	0.706	0.709	0.001
	MK5	112	5	3.582	1.398	0.732	0.721	0.724	-0.016
	T132	112	3	1.285	0.419	0.196	0.222	0.223	0.114
	KWM12	111	10	4.502	1.734	0.748	0.778	0.781	0.039
	EV37	94	12	5.775	1.992	0.713	0.827	0.831	0.138
	T80	111	8	4.093	1.602	0.712	0.756	0.759	0.058
	MK3	111	8	2.993	1.417	0.685	0.666	0.669	-0.028
	T142	112	5	2.217	1.109	0.563	0.549	0.551	-0.025
	T153	112	2	1.392	0.455	0.304	0.282	0.283	-0.078
	MK8	110	8	2.718	1.405	0.618	0.632	0.635	0.022

Columns from left to right include: Marker name, sample size, number of alleles, number of effective alleles, information index, observed heterozygosity, expected and unbiased expected heterozygosity, and fixation index

Table S2.3. Hardy-Weinberg.

Locus	Pop1	Pop2	Pop3
E12	0.918	0.044	0.620
MK6	0.755	0.729	0.078
T108	0.628	0.890	0.037
T111	0.915	0.815	0.031
T117	0.115	0.962	0.931
T128	0.010	0.431	0.827
T66	1.000	0.333	0.931
T98	0.925	0.995	0.839
D22	0.225	0.680	0.840
F10	0.865	0.070	0.948
T138	0.000	0.953	0.269
T141	0.847	0.003	0.792
T87	0.256	0.742	0.878
T91	0.598	0.312	0.732
MK9	0.011	0.293	0.611
MK5	0.528	0.670	0.944
T132	0.567	0.826	0.449
KWM12	0.311	0.707	0.861
MK3	0.960	0.944	0.000
T142	0.001	0.424	0.365
T153	0.335	0.146	0.412
MK8	0.416	0.186	0.723

All 22 markers used to estimate pairwise relatedness among males. No deviation from Hardy-Weinberg was observed throughout the three clusters.

Mitochondrial haplotype diversity

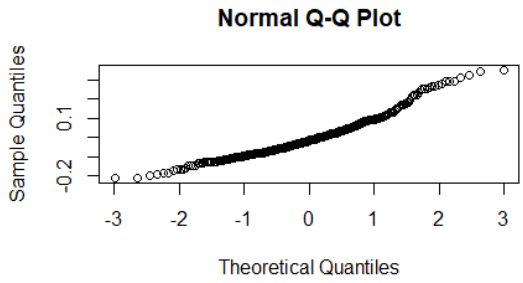
Table S2.4. mtDNA haplotypes.

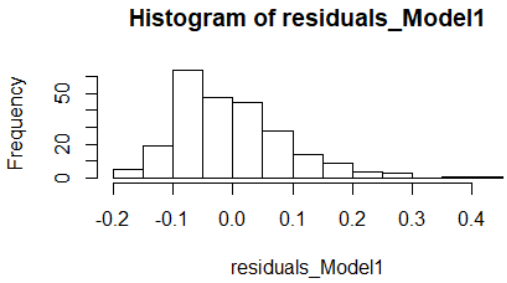
Haplotype	%
A	39.9
C	4.3
D	5.1
E	33.3
H	17.4

Distribution of haplotypes among the males (focal males and their associates) included in the study.

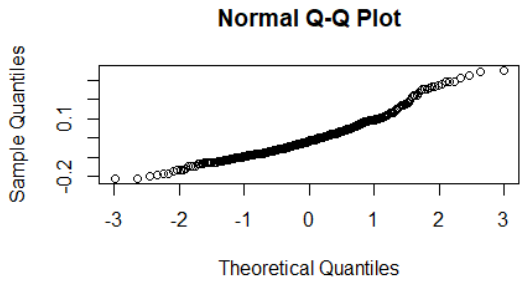
Model validation

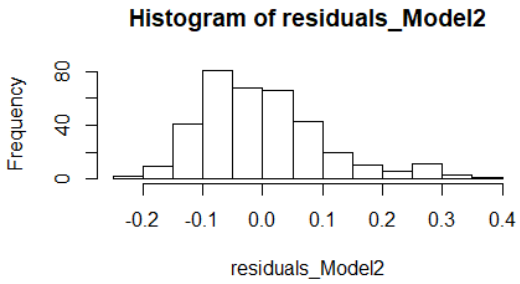
Model 1: Q-Q plot and histogram of residuals



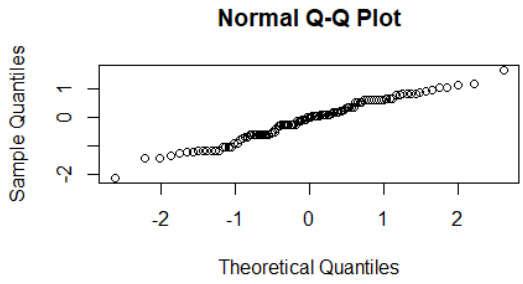


Model 2: Q-Q plot and histogram of residuals





Model 3: Q-Q plot and histogram of residuals



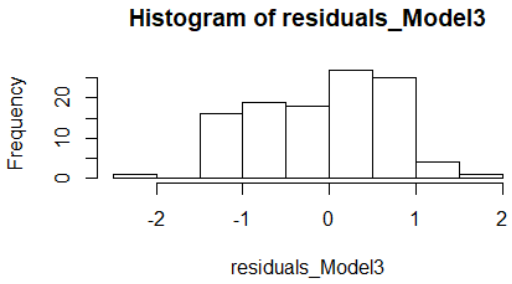


Table S2.5. Variance inflation factors (VIF) test for collinearity.

	Δage	***r-value***	***Shared haplotype***	***HWI adolescent***
Δage	1.000	-0.010	0.029	-0.173
r-value	-0.010	1.000	0.057	0.078
Shared haplotype	0.029	0.057	1.000	0.084
HWI adolescence	-0.173	0.078	0.084	1.000

Correlations of the variables included in our models.

Variance inflation factors

Δage: 1.032861

r-value: 1.008692

Shared haplotype: 1.011709

HWI adolescence: 1.044782

“A brother may not be a friend, but a friend will always be a brother.”

– Benjamin Franklin

3. Cooperative Partner Choice in Multi-level Dolphin Alliances

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Keywords: Cooperation, Biological market, Partner choice, Kin selection, Multi-level society, Male alliance

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Carried out laboratory work: L. Gerber, S. Wittwer

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Wrote the manuscript: L. Gerber

Edited the manuscript: M. Krützen, S.L. King, S.J. Allen, R.C. Connor, S. Wild, S. Wittwer, K. Holmes, W.B. Sherwin, E.P. Willems

Abstract

Investigations into cooperative partner choice should include consideration of both potential and realised partners, allowing for the comparison of traits across the available 'biological market'. Male Indo-Pacific bottlenose dolphins form multi-level alliances that can last several decades. Second-order alliances of 4-14 cooperating males are the core social units, from within which pairs and trios of males form first-order alliances to sequester individual females during so-called consortships. We examined alliance partner choice on the level of individual males by comparing social bond strength, relatedness and age similarity of potential and realised partners in two separate age periods: (*i*) adolescence (8-14 years old), when second-order alliances are formed from all available associates, and (*ii*) adulthood (15+ years old), when first-order allies are selected from within established second-order alliances.

Social bond strength during adolescence predicted second-order alliance membership in adulthood. Within these second-order alliances, non-mating season social bond strength predicted first-order partner preferences during consortships. Moreover, the importance of social bond strength during adolescence was more pronounced when the potential partner was younger than the focal male, suggesting that males prefer same-aged or older males as second-order alliance members. In contrast to most other species, kinship did not predict partner choice at either alliance level.

Our results indicate that, in the context of higher-level, polyadic cooperation, kinship becomes negligible and cooperative partner choice is instead influenced by the strength of the social bond. There is thus a striking resemblance between male dolphins, chimpanzees and humans in polyadic partner choice, where non-relatives engage in polyadic cooperative acts. To that end, our study extends the scope of taxa in which social bonds and affiliation history rather than simple kinship mechanisms explain multi-level, polyadic cooperation, providing the first evidence that such traits might have evolved independently in both the marine and terrestrial realms.

Introduction

Competition and cooperation are inherent to all forms of life, found in cellular mechanisms through to the formation and maintenance of complex societies (West and Ghoul, 2019). Both influence access to vital resources such as food, space, and mates, with the underlying mechanisms for competition explained by Darwin via natural selection and the 'struggle for existence' (Darwin, 1859). However, we still lack a complete understanding of some of the underlying proximate mechanisms of cooperation, including who cooperates with whom and when (Ghoul et al., 2017; Kay et al., 2019;

Pennisi, 2005; Rodrigues and Kokko, 2016). In many cases, there is competition for the 'best' partners, which can be thought of as a 'biological market' (McDonald et al., 2016). Such cooperative partner choice is particularly interesting when individuals have many partners from whom to choose, as is the case in large, complex societies that involve a high degree of social mixing and well-differentiated relationships.

One of the core ideas of biological markets is that individuals differ in their value as partners (Noë and Hammerstein, 1994, 1995). As in human forms of trade, individuals vary in the commodities they can offer, thereby creating competition, allowing selection to act on mechanisms improving partner choice (Bshary and Noë, 2003). Partner choice in biological markets traditionally involves two trading classes: a choosing and an offering class (Noë and Hammerstein, 1994). The choosing class is willing to 'pay' to acquire a resource that is held by the offering class. This then results in market-like situations, where agents of each class compete to make the best offers or pay the highest price to obtain a commodity. Such competition occurs in some primates, where females have an inherent interest in interacting with infants. To obtain access to infants, females without offspring (the offering class) pay by grooming mothers (the choosing class), and do so for longer when infants are rare (Henzi and Barrett, 2002; Jiang et al., 2019). Thus, the 'price' to obtain a resource is subject to supply and demand.

In settings where the outcome of cooperative acts depends on the partner's commodities (e.g., strength in a fight), individuals accrue most fitness benefits when choosing the most competent available partner (Chapais, 2006). Partner choice based on competence or another desirable commodity requires that individuals identify and potentially invest in the most valuable partners by forging social bonds, here defined as persisting affiliative relationships among individuals (Cords and Thompson, 2017; Massen, 2017). This is because high familiarity and strong social bonds often facilitate cooperation, at least in long-lived species (Barclay, 2016; Samuni et al., 2018), with known positive fitness consequences (e.g., Barbary macaques *Macaca sylvanus*, Berghänel et al., 2011; Caro, 1990; zebra finches *Taeniopygia guttata*, St-Pierre et al., 2009; reviewed in Thompson, 2019).

Along with social bond strength, kinship frequently plays a role in driving the propensity for cooperation between individuals (Bourke, 2014; Foerster et al., 2015; Silk, 2009; Strassmann et al., 2011). Relatives share genes by descent, allowing individuals to gain indirect fitness benefits when cooperating with kin (Hamilton, 1964). Interestingly, complex societies with differentiated social relationships among individuals, such as those in which supportive coalitions are formed to gain access to resources, are observed more frequently in species where average relatedness among group members is low and availability of close kin limited (Lukas and Clutton-Brock, 2018). This suggests that cooperative partner choice in such societies may be based on traits other than relatedness.

Male alliances are particularly interesting in the context of partner choice, since males must cooperate, rather than compete, to gain access to females (Díaz-Muñoz et al., 2014). Alliance formation in bottlenose dolphins (*Tursiops* spp.) is intriguing because kin selection appears to explain partner choice in some populations (Diaz-Aguirre et al., 2018; Parsons et al., 2003) but not in others (Möller et al., 2001; Wells, 2014), while complexity of alliances varies (Connor et al., 2000b). Male Indo-Pacific bottlenose dolphins (*T. aduncus*, 'dolphins' hereafter) in Shark Bay form nested alliances in an unbounded social network with high fission-fusion dynamics (Randić et al., 2012). Alliance membership is pivotal for male fitness, since non-allied males father no or very few offspring (Krützen et al., 2004a). Second-order alliances, the core male social unit, can last for decades and comprise up to 14 adult males, within which two to three males cooperate in first-order alliances to sequester single oestrus females in events known as consortships (Connor and Krützen, 2015). First-order alliances vary in composition and stability, with males showing clear preferences for particular individuals when forming first-order alliances within their second-order alliances (Connor et al., 2001; Connor and Krützen, 2015). Attacks from other alliances in attempts to steal a consorted female are defended on both alliance levels (Connor and Krützen, 2015). Allied males associate throughout the year, despite the fact that mating is markedly seasonal (Mann et al., 2000a; Smolker et al., 1992).

To date, the effect of relatedness on male alliance formation on the two alliance levels in Shark Bay remains unclear. Based on group-level relatedness patterns, previous work found that small second-order alliances consisted of more relatives than expected by chance, while a large one did not (Krützen et al., 2003). However, the alliances investigated in this study were the extremes in terms of size, as second-order alliance size in this population follows a continuum (Connor and Krützen, 2015). Furthermore, group-level analyses of relatedness are inherently problematic. Recent work, based on the individual level, showed that associations of adolescent male dolphins correlated with relatedness and that the persistence of social bonds when males transitioned from adolescence into adulthood was determined by age similarity and association history, but not kinship (Gerber et al., 2020). Thus, familiarity and age similarity appear to be more valuable traits sought for in alliance members than relatedness.

Nevertheless, two crucial details about the ontogeny of male alliance formation remain unclear. First, it is unknown whether the previously reported absence of a kinship signal is merely due to the absence of relatives within the pool of potential allies as a result of the slow life histories and singleton births (Connor and Krützen, 2015), or if males choose second-order alliance members independent of genetic relatedness. Second, marked first-order alliance partner preferences within second-order alliances have been described (Connor et al., 2001), but the basis of such individual preferences has never been investigated. Since opportunities to mate with a female within consortships are shared

among first-order alliance partners, males are expected to prefer relatives in order to maximise their evolutionary fitness.

As males are assumed to offer and seek similar commodities, we cannot identify 'choosing' nor 'offering' classes, as is usually the case when investigating biological markets. However, males still need to choose with whom they cooperate from their pool of available allies. This creates a market-like situation, with only some individuals being chosen. For this study, we identified and characterised the entire pool of potential allies on an individual level. The aims were twofold: first, to compare traits (relatedness, social bond strength, age difference) between those males that were chosen as second-order alliance members, and all those that were available but were not selected during the adolescence period, when second-order alliances are not yet established; and second, to compare these traits between preferred and non-preferred first-order partners from within established second-order alliances during adulthood.

Materials and Methods

Study population

Our study is based on long-term behavioural and genetic data collected on wild dolphins in eastern Shark Bay, Western Australia. Data collection in the form of boat-based surveys on this population started in 1984 (Connor and Krützen, 2015). A 'survey' is a minimum five-minute observation of group size and composition, as well as predominant behaviour and GPS location (Smolker et al., 1992). Tissue samples for genetic analyses have been obtained regularly since 1997 using a remote biopsy system designed for small cetaceans (Krützen et al., 2002).

In this study, we were interested in how relatedness, age and social bond strength differ between individual males and their chosen allies and their pool of potential, but non-chosen males from two age periods: (*i*) adolescence, when males are 8-14 years old and second-order alliances are first formed from their pool of associates; and (*ii*) adulthood, when males are 15 years and older and successfully consorting females in first-order alliances from within their established second-order alliances.

Identification of chosen second-order alliance members and non-chosen males

The identification of second-order alliances and their constituent members was crucial to addressing the aims of our study. Male alliances are defined both by their association indices (equal to or greater than 0.2 for second-order alliance members) and their functional behaviour, cooperating in the herding and defense of females (King et al., 2018). To confirm second-order alliance membership of adult males, *i.e.*, males fifteen years and older, we calculated association indices and carried out a hierarchical clustering analysis (as descried in King et al., 2018). More information on the identification of second-order alliances and their members is provided in the supporting information.

We calculated age difference, home range overlap and association rates for 25 'focal males' for which we knew second-order alliance membership as adults. However, we based these analyses on their time as adolescents, *i.e.*, before alliances were formed, enabling us to identify the individual pools of non-chosen males at that time period. It also allowed us to compare traits of non-chosen males to the ones of their chosen second-order alliance members. To quantify association rates, we calculated Simple Ratio Indices (SRIs) based on 5-minute survey data in the R environment v3.6.2 using *asnipe* (Farine, 2013). To reduce uncertainty in the social network, we only included males with at least 20 survey records, independent of whether they were a focal male or not. Information on SRI calculations and age estimations is detailed in (Gerber et al., 2020).

We excluded males from the pool of non-chosen males if they were more than eight years younger than the focal or had disappeared before the focal male reached adulthood. We also excluded males that were never seen in association (SRI = 0) and had a home range overlap of less than 30% with the focal male (details on this restriction are provided in the supporting information).

Choice of second-order alliance members during adolescence

We built a binomial GLMM in R using *lme4* (Bates et al., 2015) to test if the choice of second-order alliance members by the focal males once adult, was influenced by relatedness, age difference or social bond strength (SRI) during adolescence (Table S3.1, second-order GLMM). In contrast to our previous study (Gerber et al., 2020), we entered relative age differences instead of absolute age differences into our model. This allowed us to test if focal males consistently preferred older (age difference in years is negative) or younger (age difference is positive) males. To explore the effect of relatedness on the choice of second-order alliance members, we estimated pairwise relatedness between the focal males and their chosen second-order alliance members and non-chosen males from 9,991 high-quality biallelic single nucleotide polymorphisms (SNPs). Pairwise relatedness estimates were calculated using the TrioML estimator (Wang, 2007) in *Coancestry* v1.0.1.9 (Wang, 2011). A detailed laboratory protocol including bioinformatics filtering steps is provided in the supporting information.

In the second-order GLMM, we included whether males were chosen as second-order alliance members as adults or not as a dichotomous dependent variable (yes/no). Social bond strength during adolescence, age difference, and pairwise relatedness between the focal males and their chosen alliance members and non-chosen males were entered as explanatory variables, including any interactions among them. To achieve model convergence and to facilitate the calculation and interpretation of interaction terms, we applied the 'scale' function in R on the age differences that ranged from -30 to +8 years. We subdued the very large positive skew in relatedness values, spanning several orders of magnitude, by adding 1 followed by a log-transformation. To account for the dependency structure of our sample (dyadic data with repeated measures on the focal males and their potential and chosen members), we included the focal male's individual ID code and the ID codes of chosen members and non-chosen males as random effects in the model. Summary statistics, including the *P*-values for statistical significance from Wald Z-tests, were obtained using the *car* package (Fox and Weisberg, 2019).

In addition to the second-order GLMM, to assess in more detail whether males chose their second-order alliance members based on relatedness as predicted by kin selection, we modelled random second-order alliances and compared average relatedness values of focal males in these random

alliances to their actual values based on their chosen second-order alliance members (R script in the supporting information). For each focal male, we simulated 1,000 possible second-order alliances equal in size to its observed number of second-order alliance members. We did this by randomly drawing from the male's pool of potential members (e.g., 1,000 sets of six randomly drawn males for a male that had six observed second-order alliance members). Subsequently, we calculated the average relatedness of the focal males to their randomly chosen second-order alliance members for each of the 1,000 simulated alliances, as well as to their chosen second-order members. Lastly, we log-transformed the simulated values after having added 1 and compared the mean of the averaged and transformed simulated relatedness values to the observed and transformed mean using a two-tailed paired t-test.

Preferred and non-preferred first-order partners of adult males

We identified preferred and non-preferred first-order partners by calculating how often a male consorted with a specific second-order alliance member (joint consortships) and how many times they did not (separate consortships). We were interested in whether males had the strongest social bonds to their preferred first-order alliance partners outside the mating season. As male dolphins are observed together year-round and in the majority of alliance sightings, with no consorted females (Fig. S3.3), social bond strength based on association patterns provides an independent measure. To achieve this, we calculated non-mating season SRIs among second-order alliance members from survey data collected between January and July (2001 to 2018), deliberately excluding the consortship peaks between August and December (Connor et al., 1996 and Fig. S3.3). We had to exclude consortships collected in 2009, 2012 and 2018 because non-mating season data was not collected during these years. As consortships can occur all year, we further excluded all surveys that were connected to a consortship outside the mating season. Thus, we avoided the inclusion of any consortship associations to measure social bond strength.

We built a binomial GLMM in which we entered the binomial denominator consisting of number of joint and separate consortships between second-order alliance members as a dependent variable (Table S3.1, first-order GLMM). Explanatory variables were non-mating season SRIs, pairwise relatedness, and relative age difference in years. Random effects included the focal male's ID code, ID code of their second-order alliance members, as well as second-order alliance code. Scaling and transformation of data and calculations of *P*-values were carried out as described for the second-order GLMM.

Results

Choice of second-order alliance members during adolescence

We present data on 25 focal males, belonging to five different second-order alliances (Fig. S3.1 and Table S3.4). The focal males had an average of 10.4 ± 3.4 s.d. second-order alliance members as adults which were chosen from an average pool of 54.2 ± 14.3 available but non-chosen males as adolescents (Table S3.4). Genetic data were available for all chosen alliance members and for 65.8% ± 14.7 of non-chosen males. Average relatedness of all focal males to their respective pool of chosen members and non-chosen males was generally low (average r = 0.0173 ± 0.0090, average r chosen = 0.0172 ± 0.0120, average r non-chosen = 0.0173 ± 0.0102, maximum r across all focal males = 0.2001 ± 0.1339, Fig. 3.1).

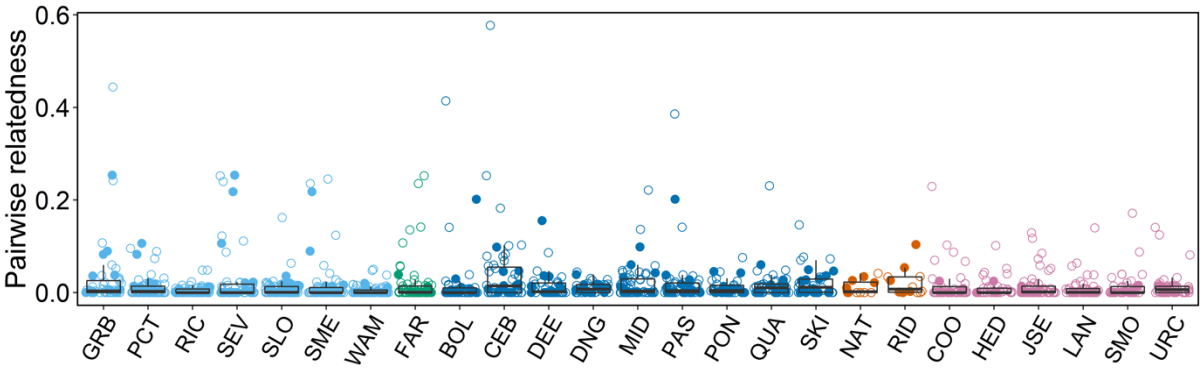

Figure 3.1. Distribution of dyadic relatedness values of each focal male and his chosen second-order alliance members and non-chosen males. Boxplots represent the upper and lower quartiles ± 1.5 interquartile range as demarked by the whiskers. Colours correspond to second-order alliances. Filled circles denote an individual's chosen second-order alliance members, the non-filled circles non-chosen males.

A binomial GLMM to quantify the likelihood of second-order alliance formation between male dyads (second-order GLMM) indicated there was a significant interaction between the relative age difference and social bond strength during adolescence between a focal and its potential allies (odds ratio = 2.53e-16, z = -2.688, *P* = 0.007, Fig.3. 2, Tables 3.1 and S3.2). This suggests that the positive effect of social bond strength during adolescence on second-order alliance member choice was modulated by relative age, as similarly aged and older potential members were more likely to be chosen by the focal at lower SRI values than younger potential members. Conversely, compared to the social bonds with older males, those with younger males had to be stronger for these younger males to be chosen as alliance members (Fig. 3.2). Generally, the majority of chosen second-order

members were of similar age to the focal males (Fig. S3.2 and Table S3.3). Further, our model showed that relatedness did not influence second-order alliance member choice (odds ratio = 3.08e-6, z = -1.453, *P* = 0.146).

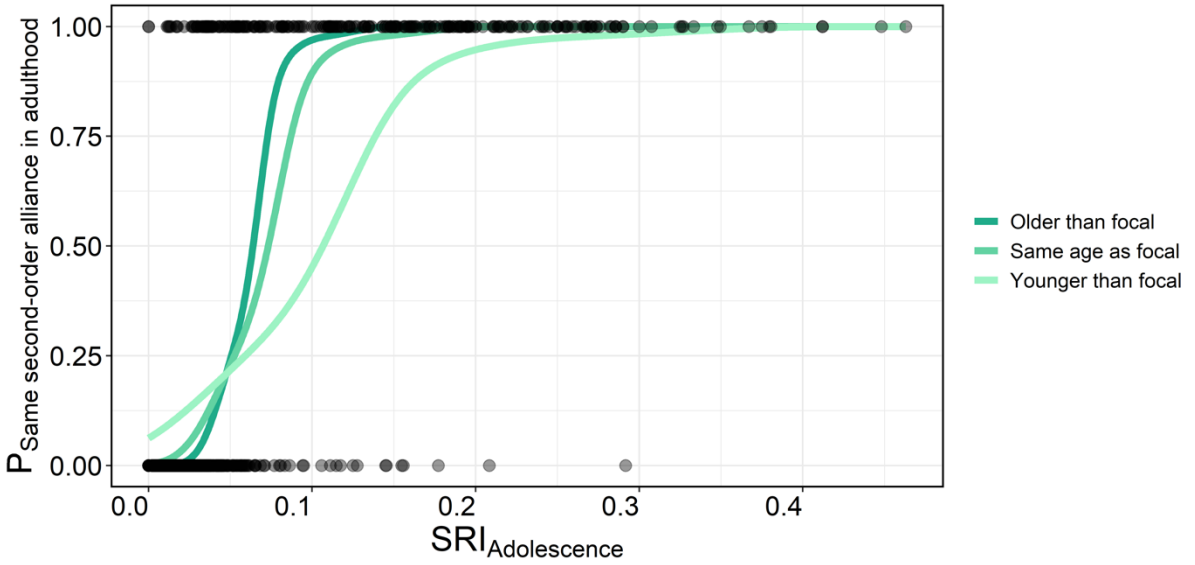

Figure 3.2. Interaction effect between social bond strength during adolescence (estimated from Simple Ratio Indices, SRI) and age difference on second-order alliance membership in adulthood. Focal males were more likely to be second-order alliance members in adulthood with males they associated with more often during adolescence, denoted by higher SRI values. However, older males and those of the same age were more likely to be chosen at lower SRI levels compared to younger males.

The finding that second-order member choice is not kin-biased was supported by our additional simulations. The transformed mean relatedness of the individual focal males' simulated alliances did not differ from the observed transformed mean relatedness between the focal males and their chosen second-order alliance members (two-tailed paired t-test, N = 25, t = -1.26, df = 24, *P* = 0.2198, Fig. 3.3).

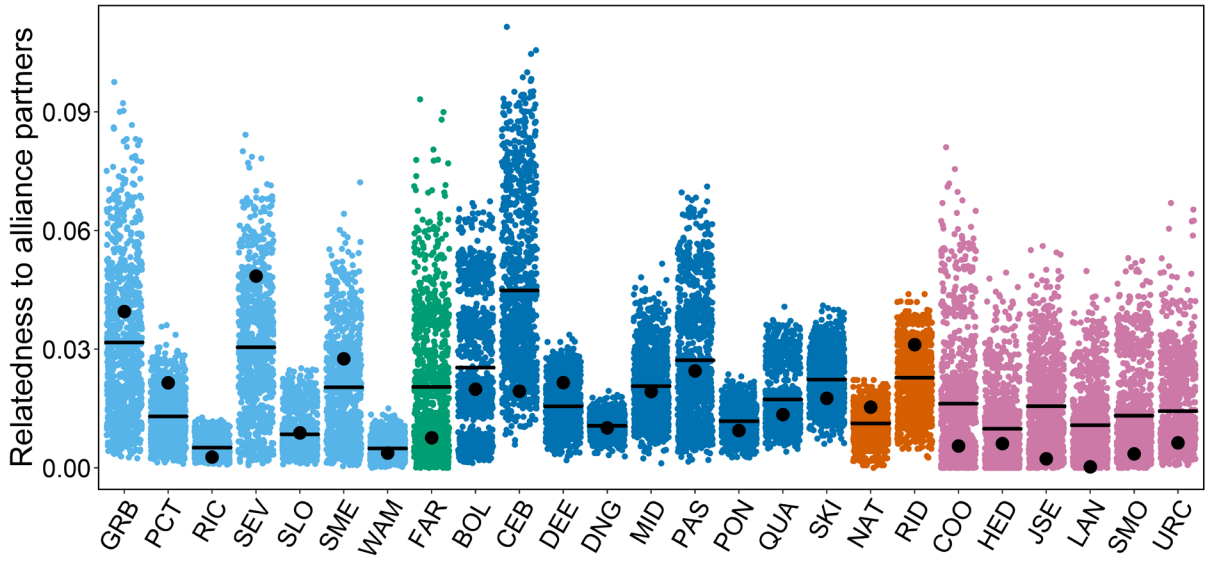

Figure 3.3. Scatter plot showing the distributions of average relatedness of focal males to randomly formed second-order alliances obtained from simulations. The mean of 1,000 simulations per male is represented by the black line. The mean relatedness value of a focal male to its actual, chosen second-order alliance members is displayed as a black circle. Different colours denote second-order alliance membership.

Preferred and non-preferred first-order partners of adult males

To explore first-order partner preference of adult males, we identified 53 well-known adult male dolphins (Table S3.5). Each male was observed in 160 ± 93 (min = 46, max = 389) surveys on average, of which 54 ± 30 (min = 21, max = 140) surveys were conducted outside of the mating season. For these males, we tested whether non-mating season social bond strength, relatedness, and age similarity predicted first-order alliance partner preference during consortships within their respective second-order alliances. The 53 males were members of six different second-order alliances (Fig. S3.1) for which genetic data were available for all members. Each of the males had on average 10.8 (± 3.2 s.d.) second-order alliance members as potential first-order alliance partners (Table S3.5). Average relatedness of the 53 males to their second-order alliance members was low (r = 0.023 ± 0.051, Fig. 3.4).

A GLMM on the first-order alliance level (first-order GLMM) revealed that partner preference of adult males on the first-order alliance level was significantly affected by social bond strength in the non-mating season, as shown by the positive correlation between non-mating season SRIs and the denominator of joint versus separate consortships (N = 482, odds ratio$_{SRI}$ = 1.23e5, z_{SRI} = 38.622, P_{SRI} < 0.0001, Table 3.1 and Table S3.2). Relatedness and age similarity did not influence the denominator of joint and separate consortships (odds ratio$_{kin}$ = 2.82, z_{kin} = 1.112, P_{kin} = 0.266, odds ratio$_{age}$ = 1.09,

z_{age} = 0.781, P_{age} = 0.435) and, thus, did not affect partner choice on the first-order alliance level. All interactions were non-significant and therefore not included in the first-order GLMM model reported here.

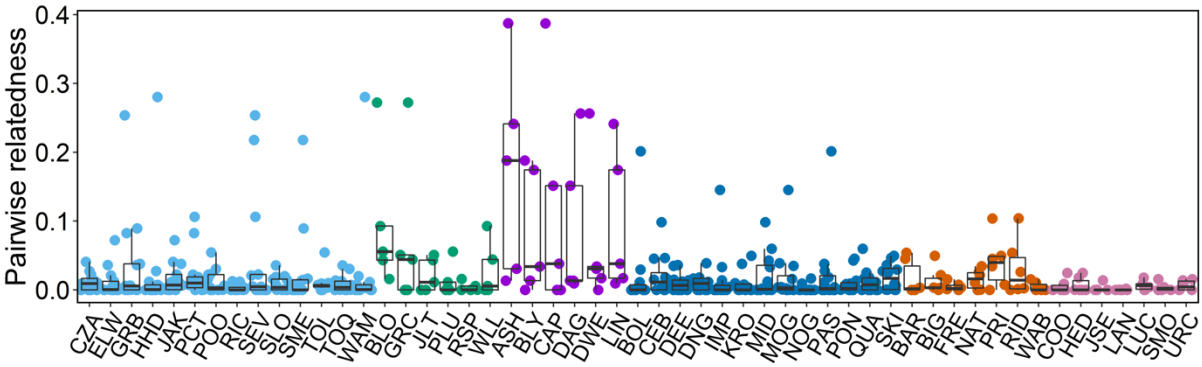

Figure 3.4. Distribution of relatedness values of 53 males to their second-order alliance members, representing their pool of males available with whom to form first-order alliances. Individual data points indicating relatedness between the 53 males and their second-order alliance members are provided as filled circles, with colours representing second-order alliance membership.

Table 3.1. Results from the GLMMs investigating second-order alliance member choice and first-order partner preferences.

	Exp(B)		***2.5%***		***97.5%***		***P-value***	
	second-order	first-order	second-order	first-order	second-order	first-order	second-order	first-order
Intercept	1.34e-5	6.39e-3	4.60e-7	2.88e-3	3.88e-4	1.42e-2	**<0.0001**	**<0.0001**
Bond strength†	1.12e58	1.23e5	8.19e41	6.77e4	1.53e74	2.23e5	**<0.0001**	**<0.0001**
ΔAge	5.48	1.09	1.39	0.878	21.54	1.35	**0.015**	0.266
Relatedness	3.08e-6	2.82	1.13e-13	0.45	83.92	17.5	0.146	0.435
ΔAge*Bond strength	2.53e-16	n.a.	1.08e-27	n.a.	5.98e-5	n.a.	**0.007**	n.a.

Exponentiated fixed effects (Exp(B)) representing odds ratios, lower and upper confidence bounds (2.5% and 97.5%) and *P*-values of the second-order GLMM and the first-order GLMM investigating the effect of pairwise relatedness, age similarity, and SRI on choices of first-order partners and second-order members. †SRI during the focal male's adolescence for the model concerning second-order alliances, non-mating season SRI between males for first-order alliance partner choice. Age*Bond strength denotes the interaction term between age difference and adolescence social bond strength on the level of second-order alliance member choice. Values in bold denote statistical significance ($P < 0.05$).

Discussion

We employed an individual-based approach to investigate partner choice on two levels of alliance formation, spanning two age periods in male Indo-Pacific bottlenose dolphins. The main factor influencing choices at both the first- and second-order alliance levels was social bond strength (Table 3.1). Social bond strength during adolescence foreshadowed second-order alliance memberships in adulthood and was of particular importance when future allies were younger than the focal males. In adult males, social bond strength during the non-mating season affected first-order alliance formation, whereby males with stronger bonds outside of the mating season tended to favour consorting together. Opposed to what is observed in most other species (Smith, 2014), relatedness between males did not affect choices at either alliance level, while age similarity influenced the choice of second-order members only.

Social bond strength thereby appears pivotal in influencing choice of allies across both alliance levels. This is expected if cooperation among males is based not on kin-selection but mechanisms such as by-product mutualism and reciprocity, where market forces will still apply (Connor, 2010). During bond formation individuals have ample time to learn about the compatibility and reliability of potential allies. Compared to female dolphins, males invest more time in social activities from an early age (Krzyszczyk et al., 2017), suggesting that early social bond formation is crucial for males. Social bond strength is generally linked to cooperation (Carter and Wilkinson, 2013; Molesti and Majolo, 2016; St-Pierre et al., 2009), but has also been found to predict male coalition formation across taxa (Berghänel et al., 2011; Gilby et al., 2013; Young et al., 2014b). During adolescence, male-male encounters may enable the assessment of each other's quality and compatibility as future allies, and promote competition over the best allies in the alliance market (Hammerstein and Noë, 2016).

Our results suggest that males prefer those individuals with whom they were closely bonded during adolescence as second-order alliance members, perhaps because they are already familiar with each other's behaviour (Cronin, 2012; Schino and Aureli, 2009a). Social bonds forged during adolescence may facilitate cooperative herding behaviours between second-order alliance members in adulthood, since males engage in consortship-like behaviours, play-herding for example, when adolescent (Connor et al., 2000b). Similarly, non-mating season social bond strength correlated with first-order partner preference in adulthood. This finding could be explained by a greater propensity to cooperate in risky tasks with closely bonded males, as previously documented in male chimpanzees (*Pan troglodytes*, Watts and Mitani, 2001). During the mating season, adult male dolphins fight with males from other alliances over access to females, entailing the risk of injury (Connor and Krützen, 2015; Connor et al., 2011).

Social bonds among individuals can often be explained by homophily, *i.e.*, the propensity to form social bonds with individuals of similar phenotypes. Homophily in age or rank (Mitani, 2009; Silk et al., 2006; Silk et al., 2012; Silk et al., 2010a), relatedness (Smith, 2014), tool use (Bizzozzero et al., 2019), and personality (Massen and Koski, 2014) can influence social bond strength. We found that the majority of chosen second-order alliance members were of similar age to the focal male. Age-based patterns of association are observed across many taxa, from blacktip reef sharks (*Carcharinus melanopterus*, Mourier et al., 2012) to chimpanzees (Mitani et al., 2002b). Frequent associations among individuals close in age may stem from shared social interests (Mitani et al., 2002b) and similar energy budgets (Ruckstuhl and Neuhaus, 2001). Considering the durability of second-order alliances, shared interests due to similar physical needs might facilitate the maintenance of cooperation and reduce the number of conflicts between alliance members. Indeed, common interests are hypothesised to limit cheating and exploitation among cooperating individuals (Hammerstein and Noë, 2016), thereby leading to stable patterns of cooperation.

Focal males showed a preference for older or same-aged males, reflected in the lower bond strength prerequisite to choosing them as second-order alliance members compared to younger males (Fig. 3.2). Young males may have less consortship experience and likely lack the physical strength of older males. Males might choose younger males as alliance members only if older or same-aged males are not available, and only those with whom they are familiar. However, as in chimpanzees (Sandel et al., 2020), older males might be desired as second-order members but are likely scarce commodities, as the majority will already belong to a second-order alliance. It is unlikely that such males would leave their second-order alliance members to join a younger male, or that established second-order alliances will take up less experienced adolescent males. However, old males that have lost alliance members may be available. Indeed, such males have been observed joining younger males that were coalescing into a second-order alliance rather than remaining alone (Connor and Krützen, 2015). With adult males already belonging to second-order alliances and younger males even less experienced than the adolescent focal males, adolescents might not have options other than forming alliances with similar-aged males in need of alliance members. Therefore, our finding that males form second-order alliances with similarly aged males might be explained by the population's demography.

Relatedness did not influence first-order partner preference or second-order member choice. This may seem surprising as male dolphins cooperate in order to gain fertilisations, an indivisible resource (Díaz-Muñoz et al., 2014). However, a species' social system and the population's demography may not allow for kin-based cooperation (Sherman, 1981). This has been observed in male chimpanzees (Mitani et al., 2002b), male Galapagos hawks (*Buteo galapagoensis*, Faaborg et al., 1995), and cooperatively breeding choughs (*Corcorax melanorhamphos*, Heinsohn, 1991), in which relatives were

preferred cooperative partners but not always available. In dolphins, demographic constraints due to singleton births (Mann et al., 2000a), long interbirth intervals and a lack of reproductive skew at the population level (Krützen et al., 2004a) result in the availability of few close male relatives. This is also supported by the low average relatedness we found between the focal males and their available allies at both alliance levels (Fig. 3.1 and 3.4). Interestingly, and in contrast to what is observed in chimpanzees, male dolphins did not prefer close relatives as alliance members ($r \geq 0.25$) even when available (Fig. 3.1). Furthermore, we know of at least three cases where successive maternal brothers are in different second-order alliances (unpublished data). Skill development may thus be of sufficient importance that males maintain already developed social bonds even when a maternal half-brother becomes available.

In species that give birth to multiple offspring at once, or with high reproductive skew, multiple individuals share a set of half-siblings through either the maternal or paternal line. A larger number of shared half-siblings facilitates kin-biased alliance formation (e.g., littermates in cheetahs *Acionyx jubatus*, Caro, 1990). The social and reproductive system in Shark Bay means that individual dolphins are unlikely to have many close relatives available, impeding the formation of kin-biased polyadic alliances. Although individuals could form dyadic alliances with kin to gain indirect fitness benefits, these may be offset by the direct benefits gained through the formation of larger, polyadic alliances with non-kin. Evidence that relatedness becomes negligible in the context of higher level, polyadic cooperation can be found in both chimpanzees and humans, where relatives are preferred partners in dyadic but not polyadic settings (Nolin, 2011; Suchak et al., 2014).

Our results suggest that cooperative partner choice in the market of available potential allies is a directed, non-stochastic process and, due to the impact on fitness, male dolphins in Shark Bay value those with whom they share the strongest bonds during adolescence as allies in adulthood, independently of relatedness. Our findings bear striking analogies to what is known on polyadic cooperation in chimpanzees and humans. Owing to this, our results imply that cooperation among non-kin is not unique to primates but a common feature of complex societies, marine and terrestrial alike.

Acknowledgements

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Supporting Information

Second-order alliance identification and members

Second-order alliance membership was confirmed by calculating Simple Ratio Indices (SRI) based on five-minute survey data and a hierarchical clustering analysis (as described in King et al., 2018) as well as by documenting consortships between the males (consortships were scored based on established criteria, as outlined below). For the purpose of this study, we identified and confirmed alliance membership for a total of 58 males belonging to six second-order alliances with variable alliance sizes of 6-14 individuals (Fig. S3.1). We had to exclude five alliance members (BAK, EXF, FAR, JAA, TER) from the analyses investigating first-order alliance partner choice because they were seen fewer than 20 times in the non-mating seasons between 2001 and 2018 and, thus, their non-mating season associations were considered unreliable. Furthermore, we could not estimate the year of birth for JAA.

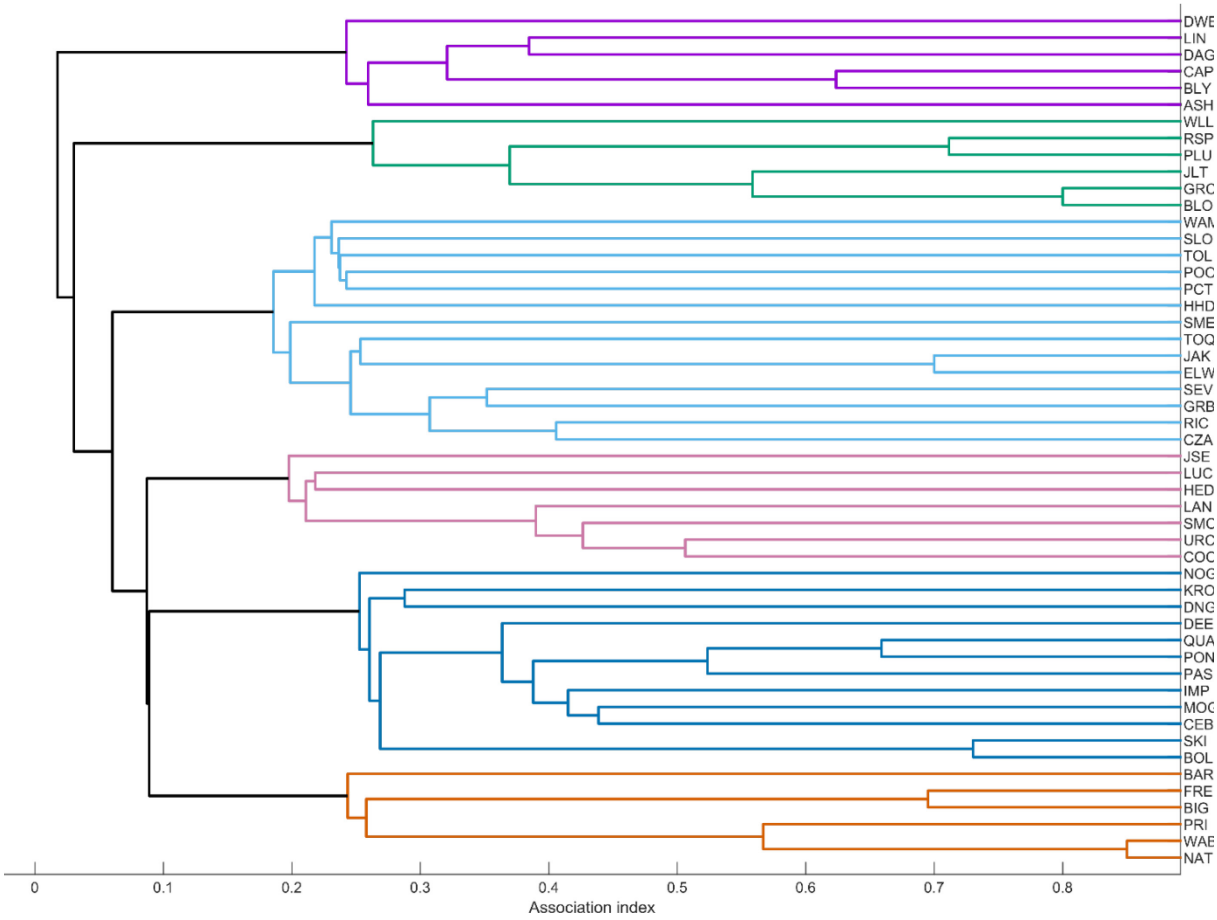

Figure S3.1. Dendrogram of the second-order alliances identified functionally via consortship data and confirmed via hierarchical clustering analysis based on survey data.

Shark Bay ethogram – consortship criteria

Consortships are coercively maintained associations between 2-3 males and one female. Consortships are scored as YES (Y), NO (N), or PROBABLE (P) for both the male subgroup and suspected female consort. Scoring the consortship as a YES for both males and female consort requires satisfaction of the following criteria:

A) The subgroup of 2-3 males + 1 female is observed with >10m separation between any other individual or group, AND

B) ONE or more of the following 6 events are observed:

1. **1hr:** Consortship subgroup is observed for at least 1 hour. At least one male from the consortship subgroup is within 10m of the suspected female throughout the 1hr observation period.

2. **Capture:** The female is captured by an alliance of males.

3. **Bolt:** Female attempting to escape by rapidly swimming ('bolting') from an alliance of males.

4. **Pops:** At least one of the males producing a vocal threat called 'pops' that induces the female to remain close.

5. **Directed Aggression:** At least one of the males directing physical threats or aggression toward the female. Aggressive behaviour described in the Shark Bay Dolphin Research Ethogram.

6. **Theft:** Teams of two alliances attempting to take a female from another alliance. In this case, the consortship is scored as a 'Y' for losing males, winning males, and female.

If A observed but B is not observed, the consortship should be scored as a 'P' if:

- > 10m separation, one female, >30 min but < 1hr observation ('P' for males & female)

Model specifications

Table S3.1. Model specifications for Models 1 (second-order GLMM) and 2 (first-order GLMM).

Model	
1	second-order members y/n ~ r + Δage*SRI + (1|ID_F) + (1|ID_C)
S1	second-order members y/n ~ Δage + Δage^2 + (1|ID_F) + (1|ID_C)
2	separate : joint consortships ~ $SRI^{\dagger}$+ Δage + r + (1|ID) + (1|ID_P) + (1|Alliance)

Model specifications for Models 1 (second-order GLMM) and 2 (first-order GLMM) investigating partner choice on the second-order and first-order alliance level. Of all possible interactions, only the one between age difference and social bond strength on the level of second-order member choice was significant and thus, included. Both binomial GLMMs included relative age difference (Δage), social bond strength (SRI), and relatedness (r). We log-transformed the relatedness values after adding 1 and applied the 'scale' function to the age difference before entering them into the Models.

The second-order GLMM investigated whether two males became members of the same second-order alliance or not (second-order members y/n). Social bond strength in the second-order GLMM was the SRI between the focal and a male from its cast during the focal male's adolescence. The * denotes the interaction effect between age difference and social bond strength. The random effects of the second-order GLMM consisted of the three letter ID codes of the focal male (ID_F) and the males from their cast, consisting of chosen alliance members and non-chosen males (ID_C).

Model S1 is a post-hoc GLMM analysis based on the results of the second-order GLMM in which we investigated if males were more likely to form second-order alliances with similarly-aged males. Model S1 contains relative age difference as linear variable as well as its quadratic term. Also here, the scale function was applied on Δage.

In the first-order GLMM, the binomial denominator consisted of how often two second-order alliance members were observed in joint or separate consortships (separate : joint consortships), allowing us to explore first-order alliance partner choice. In this model, social bond strength ($SRI^{\dagger}$) was the non-mating season SRI between second-order alliance members and included second-order alliance membership (Alliance) as a random factor.

Detailed results of first- and second-order GLMMs

Table S3.2. Results of the second-order GLMM and first-order GLMM (Table S3.1).

	B		*S.E.*		*z-value*		*p-value*	
	second-order	first-order	second-order	first-order	second-order	first-order	second-order	first-order
Bond strength†	133.663	11.718	18.957	0.303	7.051	38.622	**<0.0001**	**<0.0001**
Relatedness	1.701	0.086	0.698	0.110	2.437	0.781	0.146	0.266
ΔAge	-12.690	1.037	8.735	0.932	-1.453	1.112	0.015	0.435
ΔAge*Bond strength	-35.911	n.a.	13.361	n.a.	-2.688	n.a.	0.007	n.a.

Results of the second-order GLMM and first-order GLMM (Table S3.1), investigating the effect of pairwise relatedness, age similarity, and SRI on ally choice on the first- and second-order level of male alliances. †SRI during the focal male's adolescence for the model concerning second-order alliances, non-mating season SRI between for first-order alliance partner choice. Age*Bond strength denotes the interaction term between age difference and social bond strength on the level of second-order alliance member choice.

Results of Model S1

To test if males chose similarly-aged males as second-order alliance members which could not be inferred from the second-order GLMM due to non-convergence, we ran a post-hoc GLMM (Model S1) in which we entered age differences as a quadratic as well as a linear term. The significant effect of the quadratic term suggests that focal males primarily formed second-order alliances with their peers (N = 1,180, Table S3.3, Fig. S3.2).

Table S3.3. Results of Model S1 investigating if males chose similarly-aged or older males as alliance members.

	B	*S.E.*	*z-value*	*p-value*	*C^B*	*2.5%*	*97.5%*
Intercept	-3.180	0.443	-7.174	**<0.0001**	0.042	0.02	0.10
ΔAge²	0.975	0.385	2.535	**0.0111**	2.65	1.24	5.63
ΔAge	-1.751	0.487	-3.596	**0.0003**	0.17	0.066	0.45

Results of Model S1 investigating if males chose similarly-aged or older males as alliance members. The significant effect of the quadratic age difference indicates that focal males were more likely to form second-order alliances with similarly-aged males.

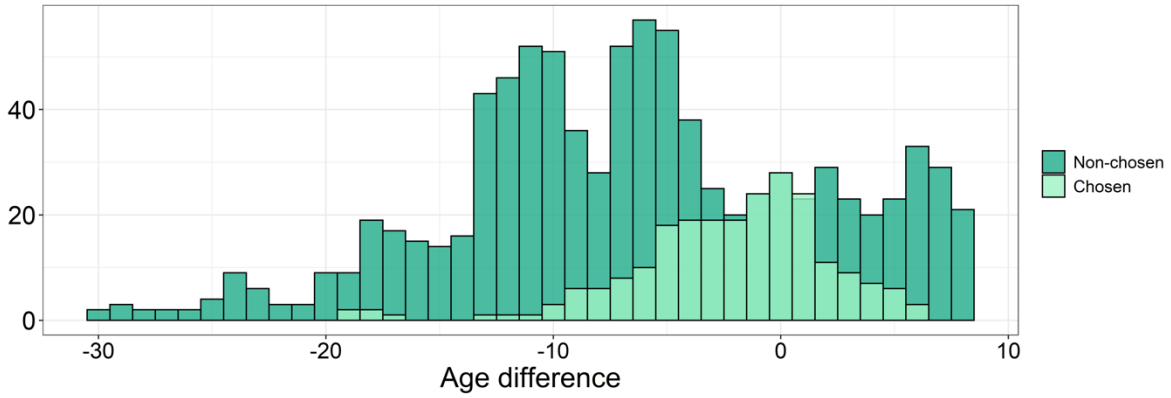

Figure S3.2. A large proportion of chosen alliance members were of similar age to the focal, while males with larger age differences were chosen less often.

Home range overlap of second-order alliance members

The smallest observed home range overlap between adolescent males who became actual second-order alliance members was 0.36. Slightly more conservative, we used 0.30 as a cut-off to exclude males as potential members based on their home range overlap. However, males with home range overlaps smaller than 0.30 but who were observed in association with the focal male at least once during the focal male's adolescence were still treated as potential alliance members for second-order alliance partner choice.

Seasonality of consortships

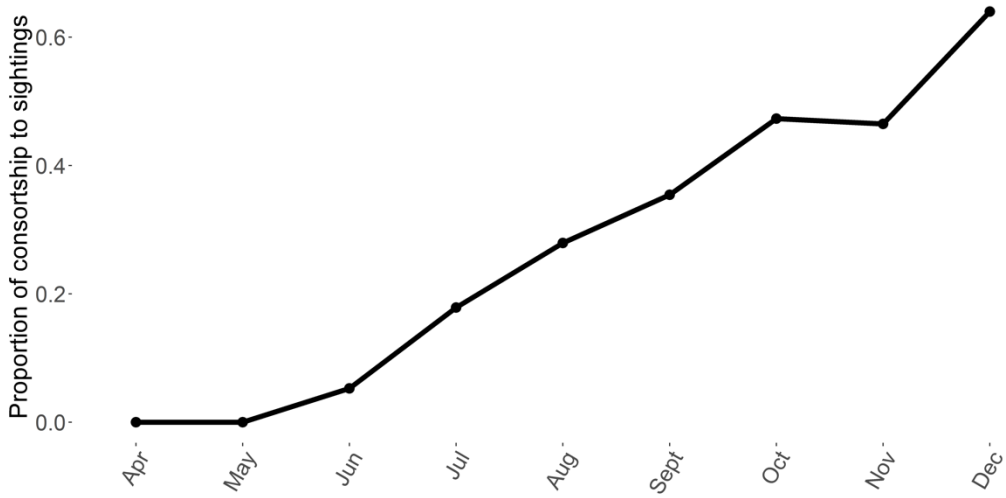

Figure S3.3. Line plot visualising the increase of consortships at the start of the mating season in August. The Y-axis contains the proportion of consortship to non-consortship sightings (during surveys) of male dolphins.

Table S4. Summary of the 25 focal males to investigate second-order alliance member choice.

Focal ID	Number of non-chosen males / thereof sampled	Number of chosen second-order alliance members
BOL	52 / 25	13
CEB	54 / 34	13
COO	50 / 39	6
DEE	53 / 25	13
DNG	49 / 23	13
FAR	67 / 57	7
GRB	55 / 40	13
HED	53 / 36	4
JSE	58 / 43	6
LAN	51 / 40	6
MID	75 / 48	13
NAT	14 / 6	6
PAS	54 / 30	13
PCT	61 / 51	12
PON	50 / 22	13
QUA	60 / 35	13
RIC	69 / 54	13
RID	14 / 6	6
SEV	54 / 39	13
SKI	52 / 25	13
SLO	79 / 63	13
SME	54 / 44	13
SMO	47 / 37	6
URC	64 / 44	6
WAM	64 / 55	12

The table provides each focal male's ID code, their number of potential, non-chosen males and how many thereof were genotyped. The last column indicates each focal male's number of actual, chosen second-order alliance members.

Table S3.5. Overview of the 53 adult focal males used to investigate first-order alliance partner choice.

second-order alliance	ID focal	Number of potential first-order alliance partners
BL	CZA	13
BL	ELW	13
BL	GRB	13
BL	HHD	13
BL	JAK	13
BL	PCT	13
BL	POO	13
BL	RIC	13
BL	SEV	13
BL	SLO	13
BL	SME	13
BL	TOL	13
BL	TOQ	13
BL	WAM	13
HC	ASH	6
HC	BLY	6
HC	CAP	6
HC	DAG	6
HC	DWE	6
HC	LIN	6
KS	BAR	14
KS	BOL	14
KS	CEB	14
KS	DEE	14
KS	DNG	14
KS	IMP	14
KS	KRO	14
KS	MID	14
KS	MOG	14
KS	NOG	14
KS	PAS	14
KS	PON	14
KS	QUA	14
KS	SKI	14
PD	BIG	5
PD	FRE	5
PD	NAT	5
PD	PRI	5
PD	RID	5
PD	WAB	5
RR	COO	6
RR	HED	6
RR	JSE	6
RR	LAN	6

RR	LUC	6
RR	SMO	6
RR	URC	6
XF	BLO	8
XF	GRC	8
XF	JLT	8
XF	PLU	8
XF	RSP	8
XF	WLL	8

Overview of the 53 adult focal males used to investigate first-order alliance partner choice, including each focal male's second-order alliance membership and size thereof, reflecting its pool of available second-order alliance members to choose as first-order alliance partners. All members of all second-order alliances have been genotyped successfully.

Modelling second-order alliances to test the influence of relatedness on partner choice

```
### MODEL OF RANDOM SECOND ORDER ALLIANCE CHOICE BASED ON SHARED RELATEDNESS ###

# LOAD FILE CONTAINING IDs (FOCAL IN COLUMN 1, POTENTIAL PARTNER ID IN COLUMN 2), R-VALUES BETWEEN IDs IN COLUMN 10, ACTUAL ALLIANCE SIZE OF FOCAL IN COLUMN 29, AVERAGE R-VALUE TO ACTUAL ALLIANCE PARTNERS IN COLUMN 30

FocalMalesSRI_LE_20190214_LMM_no0TrioML <- read.csv(YourPath:/FocalMalesSRI_LE_20190214_LMM_no0TrioML.csv", header=TRUE)

#USE AGGREGATE FUNCTION TO OBTAIN LIST CONTAINING ALL INDIVIDUALS AND THEIR SECOND-ORDER ALLIANCE SIZE

IDs <- aggregate(FocalMalesSRI_LE_20190214_LMM_no0TrioML[,30],list(FocalMalesSRI_LE_20190214_LMM_no0TrioML$ID1),mean)

#CREATE AGENTS (I.E. FOCAL MALES)

setup <- function(){

return(data.frame(id=c(IDs$Group.1), averageR=c(IDs$x)))

}

indDF <- setup()

#SPLIT UP DATASET AND CREATE A LIST CONTAINING SEPARATE DATAFRAMES OF ALL AGENTS AND THEIR POTENTIAL PARTNERS

potentialpartners <- split(FocalMalesSRI_LE_20190214_LMM_no0TrioML, FocalMalesSRI_LE_20190214_LMM_no0TrioML$ID1)

potentialpartners <- as.list(potentialpartners)

#EXTRACT R VALUES TO POTENTIAL PARTNERS (COLUMN3) AND NUMBER OF ACTUAL ALLIANCE PARTNERS (COLUMN 29) FOR EACH AGENT

ObtainrvaluesAA <- function(x){

x[,c(10,29)]

}

allRvaluesAndAA <- lapply(potentialpartners, ObtainrvaluesAA)

chooseAlly <- function(ind){

chosenPartner <- sample(x = ind$TrioML, size = mean(ind$AA), replace = FALSE) #randomly choose alliance partner

ind$partner <- mean(chosenPartner)
```

```
return(ind)

}

#EXTRACT AGENT'S ID AND AVERAGE R-VALUE OF RANDOM ALLIANCES FORMED OF EACH
INDIVIDUALS ALLIANCE PARTNERS, FEED VALUES INTO SEPARATE LIST

Form1000Alliances <- replicate(1000,lapply(allRvaluesAndAA,chooseAlly))

x <- as.data.frame(do.call("rbind",lapply(Form1000Alliances,"[",1,3)))

tmp <- data.frame(

X = x$V1,

ind=rep(1:34,nrow(x)/34000) #34 = NUMBER OF AGENTS

)

Average1000Alliances <- unstack(tmp, X~ind)

y <- apply(Average1000Alliances,2,mean)

indDF["averageRandomChoice1000"] <- y

#CARRY OUT T-TEST TO SEE IF MEAN OF ALL AVERAGE R-VALUES OF 1000 RANDOM ALLIANCES
DIFFERS FROM OBSERVED AVERAGE R-VALUE TO ALLIANCE PARTNERS

t.test(indDF$averageR,meansSimulated, paired = TRUE, alternative = "two.sided")
```

Quality filtering to identify high-quality SNPs

To identify high-quality biallelic SNPs for accurate relatedness estimation, we aligned the quality filtered reads against a *T. truncatus* reference assembly (GenBank: GCA_001922835.1 (O'Leary et al., 2016) using bowtie2 v2.2.6 (Langmead and Salzberg, 2012) with the 'very-sensitive' preset. Variant calling was performed using GATK v4.1.1.0 by first generating per-individual g.vcf files with HaplotypeCaller and subsequent joint variant calling using CombineGVCFs. We hard-filtered the resulting 54'854 raw SNP variants with vcftools (Danecek et al., 2011) and obtained our final 9,991 biallellic SNP loci based on a phred quality score over 30, sequencing depth per locus of at least five, per locus coverage in over 50% of individuals, minor allele count of 3, and minimal distance between SNPs of 100kb.

ddRAD laboratory protocol

Genomic DNA was extracted from small tissue biopsy samples using the Gentra Puregene Tissue Kit (Qiagen). Subsequently, we quantified our DNA extracts using a Qubit 1.0 fluorometer with the Qubit dsDNA BR Assay Kit (ThermoFisher Scientific). We set up a restriction digest consisting of 250 ng of genomic DNA per individual and 20 units of MseI (New England Biolabs, 'NEB') as well as high-fidelity EcoRI (New England biolabs), followed by eluting the digested DNA twice in 18µl buffer using the MinElute PCR cleanup (Qiagen). To normalise the samples and to ensure even representation during sequencing, we used the Qubit with the dsDNA HS Assay Kit (ThermoFisher Scientific).

To each sample, we ligated differing EcoRI P1 barcode adapters in order to assign obtained reads to individuals after sequencing. MseI restriction ends were ligated to MseI P2 adapters containing degenerate bases, allowing us to detect PCR duplicates after sequencing (Tin et al., 2015). All sequences of adapters, barcodes and primers used are provided in the SI. The ligation reaction per sample was set up in a total volume of 45µl and consisted of 400 Units of T4 Ligase (NEB), 4.5µl T4 Ligase Buffer (NEB), 1.5µl MseI adapter (10µM), 1.5µl EcoRI adapter (1µM), and 150ng DNA. The ligation took place in a Veriti Thermal Cycler (Applied Biosystems) set to 23°C for one hour, followed by 65°C for 10 minutes and was cooled to 4°C at a rate of 2°C per 90 seconds.

After the ligation, the now individually-barcoded samples were pooled and size-selected. We used three AMPure bead size selections. The first two to obtain fragments in the approximate range between 200 and 500 base pairs. For this, we added 0.65 volumes of AMPure bead mix to the pooled samples to remove larger fragments and added 0.16 volumes (of the original volume) to remove small

fragments. In the third bead selection we added 1.2 volumes of AMPure beads, ensuring complete removal of the adapter dimers and eluted the DNA fragments in 28µl of ddH_2O.

The size-selected DNA was then amplified via PCR. We set up ten 30µl reactions consisting of 1.5µl primer 1 and 2 each (10µM stock concentration), 0.9µl DMSO, 15µl Phusion Mastermix 2X (NEB), 8.7µl ddH2O, and 2.4µl DNA. The reactions were transferred to a preheated (98°C) thermocycler (Labcycler, SensoQuest). Followed by an initial denaturation of 98°C for 30 seconds, the DNA was amplified in ten PCR cycles (denaturation at 98°C for 10 seconds, annealing at 65°C for 30 seconds, extension at 72°C for 30 seconds). Post PCR, the reactions were pooled and cleaned up in a single MinElute PCR cleanup column including several loading steps and eluted twice in 18µl EB buffer, yielding 36µl of amplified product.

To ensure sequencing of homologous genomic regions across the different libraries, we carried out a last size selection by gel electrophoresis on Spreadex® EL600 gels (AL Diagnostics) as described in (Greminger et al., 2014) with the following modifications: we loaded a 25µl amplified library in two separate lanes to avoid DNA overloading and ran the gel electrophoresis for 228 minutes at 120 Volt. We avoided UV exposure by visualising DNA fragments on a blue light transilluminator (Dark Reader DR46B, Clare Chemical Research) and excised fragments between 307 and 404 bp. For DNA recovery using electro elution, the packages containing the excised gel fragments were placed in the electrophoresis chamber overnight at a buffer temperature of 20°C at 90V.

We produced a total of eight libraries, consisting of 40 samples each. The libraries were sequenced in the rapid run mode on an Illumina HiSeq2500, using one lane per library, combining two libraries per run. To distinguish between samples of the same barcode but different libraries, one library was ligated to MseI adapters with an Index 6 sequence, the other to Index 12 or Index 4 (sequences are provided in the detailed laboratory protocol below).

Step-by-step ddRAD wet lab protocol

*Adapters:***Adapter P1 = *Eco*RI x 48**

P1.1 (*Eco*RI top):

5’ ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGAT 3’

P1.2 (*Eco*RI bottom):

5’ AATTATCGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT 3’

1	GCATG_EcoRI	25	CTGCG_EcoRI
2	AACCA_EcoRI	26	CTGTC_EcoRI
3	CGATC_EcoRI	27	CTTGG_EcoRI
4	TCGAT_EcoRI	28	GACAC_EcoRI
5	TGCAT_EcoRI	29	GAGAT_EcoRI
6	CAACC_EcoRI	30	GAGTC_EcoRI
7	GGTTG_EcoRI	31	GCCGT_EcoRI
8	AAGGA_EcoRI	32	GCTGA_EcoRI
9	AGCTA_EcoRI	33	GGATA_EcoRI
10	ACACA_EcoRI	34	GGCCA_EcoRI
11	AATTA_EcoRI	35	GGCTC_EcoRI
12	ACGGT_EcoRI	36	GTAGT_EcoRI
13	ACTGG_EcoRI	37	GTCCG_EcoRI
14	ACTTC_EcoRI	38	GTCGA_EcoRI
15	ATACG_EcoRI	39	TACCG_EcoRI
16	ATGAG_EcoRI	40	TACGT_EcoRI
17	ATTAC_EcoRI	41	TAGTA_EcoRI
18	CATAT_EcoRI	42	TATAC_EcoRI
19	CGAAT_EcoRI	43	TCACG_EcoRI
20	CGGCT_EcoRI	44	TCAGT_EcoRI
21	CGGTA_EcoRI	45	TCCGG_EcoRI
22	CGTAC_EcoRI	46	TCTGC_EcoRI
23	CGTCG_EcoRI	47	TGGAA_EcoRI
24	CTGAT_EcoRI	48	TTAACC_EcoRI

Table containing barcode sequences to distinguish pooled samples bioinformatically after sequencing**.**

Adapter P2 = *Mse*I
P2.1 (*Mse*I top):
5’ /5Phos/TAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC 3’

P2.2 (*Mse*I bottom):
Idx_6 GCCAAT
5’ CAAGCAGAAGACGGCATACGAGATNNNN**ATTGGC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC 3’

Idx_12 CTTGTA
5’ CAAGCAGAAGACGGCATACGAGATNNNN**TACAAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC 3’

Idx_4 TGACCA
5’ CAAGCAGAAGACGGCATACGAGATNNNN**TGGTCA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC 3’

Illumina PCR primers
ILLPCR1
5’ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG 3’

ILLPCR2
5’ CAAGCAGAAGACGGCATACGA 3’

See last page for an overview of the setup presented here

Preparation of Specialised Reagents

Adapter P1: Barcoded *Eco*RI primer combinations.

- Combine 1µl P1.1 + 1µl P1.2 (100µM stock) with 98µl ddH_2O to make 100ul of 1µM annealed, double stranded adapter stock.
- Heat to 95°C for 5 min and slowly cool to room temperature (no faster than 2°C/min).

    - Keep the adapters organised in plate format for easy use in future directions
    - Adapters can be kept at 4°C when in use but not longer than 2 weeks. For long term storage keep at -80°C.

*Adapter P2: Mse*I-bar adapter

- Combine 10µl of P2.1 + P2.2 (100µM stock) with 80µl ddH_2O to make 100µl of 10µM stock.
- Heat to 95°C for 5min and slowly cool to room temperature to anneal oligos into double-stranded adapters (not faster than 2°C/min).

    - Storage same as above.

PCR primers

- Mix 50µl of each primer (100µM stock) with 900µl ddH_2O to make a working solution containing 5µM of each primer.

- Storage same as above.

Sample Preparation

Measure DNA concentration

- Start with 15µg of DNA per library. For 50 samples this equates 300ng of DNA/sample, made up to 84µl with ddH_2O.
- The cleanup/ligation needs to be carried out in a clean PCR plate (easier for AMPure cleanup)

1. Restriction Digest

Prepare Master Mix I

- 16µl per sample
- Use low binding Eppendorf tubes due to high viscosity of the enzymes.

Reagent	1x	1x (ul)	55x (ul)
Cutsmart buffer (10x)	1x	1.6	88
*Mse*I (10,000U/ml)	20U	2.0	110
*Eco*RI (20,000U/ml)	20U	1.0	55
ddH_2O		11.4	627
Total		***16***	***880***

- Mix by vortexing and centrifuge briefly.
- Add 16µl of MM1 to each well
- This gives a total of 100µl.
- Seal plate. Vortex and spin down.
- Digest at 37°C for 7hrs and cool to RT
- Do not heat kill as this skews base composition
- 20U of enzyme digests 1mg DNA in 30min, but DNA was found to not always be digested completely
- There is uneven digestion between samples
- Before moving to the next step, cool restriction digest to RT. Alternatively, product can be stored at 4°C overnight.

MinElute PCR Cleanup

The columns clean up the Restriction digest but don't get rid of the adapter dimer. This needs to be cleaned with AMPure cleanup just before the size selection.

- Follow the manufacturer's instructions.
- Elute twice in 18µl buffer for a total elute of 36µl (incubate for 5 minutes each time).

- 1µl can be kept to run on tapestation/gel

Normalise Samples

- Determine the DNA concentration of each cleaned digest using Qubit dsDNA BR (1µl).
- Normalise the samples (100-300ng/sample is optimal)

This step is essential for ensuring even representation of samples in the sequencing.

Adapter Ligation

Defrost *Eco*RI adapters (in plate form) and *Mse*I-bar adapters (in one eppie in the -80°C freezer) in the fridge or on ice.

- Make Master Mix II (MMII)

Reagent	**1x**	**1x (µl)**	**55x (µl) Idx_XX**
T4 Ligase Buffer 10x	1x	4.5	247.5
*Mse*I Adapter (10µM)		1.5	82.5
T4 Ligase (400 U/ml)		1	55
Water + DNA		36.5	
Total		*45.0*	*7µl/sample*

- Add 7ul of MMII to each well containing 36.5µl DNA + water. Add 1.5µl of the *Eco*RI (1µM working solution) adapters to each corresponding sample well for a total volume of 45µl.
- Ligate in PCR cycler: 1hr @ 23°C. 10min @ 65°C. Cool to 4°C at a rate of 2°C/90sec.
- Keep 1µl to run on Tapestation

Pooling and Size Selection

Be aware that different fragments are obtained when carrying out the size selection in Lo-bind tubes

Before you continue:

- Leave AMPure beads on the bench for 30min to equilibrate to RT
- Make fresh 80% EtOH
- Pool half of all the individually barcoded DNA samples in one tube and mix well, do the same with the other half and carry out the bead selection on both tubes at the same time.

First bead selection to remove large fragments

- Add 0.65 volumes AMPure bead mix to the pooled samples (e.g., 65µl for a volume of 100µl).
- Mix by pipetting up and down at least 10 times.
- Incubate on the bench for 10-15min at RT.
- Place on magnetic stand to separate beads from supernatant 5min.
- Carefully transfer all supernatant to a new tube (contains all fragments <370bp!)

Second bead selection to remove small fragments

- Add 0.16 volumes (of the original volume, e.g., 16µl if original volume was 100µl) to the supernatant, mix well and incubate for 5-10 minutes at RT
- Put the plate on a magnetic stand and remove the supernatant after the solution cleared up (5 min).
- Wash the beads 2x with 200µl freshly prepared 80% EtOH while still on the magnetic stand, incubate for 1 min and carefully remove and discard the supernatant.
- Still on the magnetic stand, leave the plate to dry (with open cap) at RT for 5-10min. DO NOT OVERDRY! (lower DNA recovery rate)
- Remove the tube from the magnetic stand. Elute in 40µl (0.1X TE or ddH_2O). Mix well by pipetting up and down and incubate for 2 minutes at RT.
- Put the tube back on the magnetic stand for 3 minutes. Transfer the supernatant to a clear tube.
- Pool both supernatants into a single tube.

Third bead selection to remove adapter

- Add 1.2 volumes (of the original volume, here the eluted one, approximately 80µl) to the cleaned up DNA, mix well and incubate for 5-10 minutes at RT
- Put the plate on a magnetic stand and remove the supernatant after the solution cleared up (5 min). DO NOT DISTURB THE BEADS! (contain DNA targets)
- Wash the beads 2x with 200µl freshly prepared 80% EtOH while still on the magnetic stand, incubate for 1 min and carefully remove and discard the supernatant.
- Still on the magnetic stand, leave the plate to dry (with open cap) at RT for 5-10min. DO NOT OVERDRY! (lower DNA recovery rate)
- Remove the tube from the magnetic stand. Elute in 28µl (0.1X TE or ddH_2O). Mix well by pipetting up and down and incubate for 2 minutes at RT.
- Put the tube back on the magnetic stand for 3 minutes. Transfer the supernatant to a clear tube.
- 1µl is kept to run on the Tapestation.

PCR

Set the reaction up on ice and quickly transfer the reaction to the preheated (98°C) thermocycler. The Phusion MasterMix is added last in order to prevent any primer degradation

30µl reaction (10X)
1.5µl primer 1 (10µM)
1.5µl primer 2_index (10µM)
0.9µl DMSO
15µl Phusion Mastermix 2X, NEB
2.4µl size selected DNA and 8.7µl ddH_2O for 5X diluted PCR

10 PCR cycles

Initial Denat.	98°C 30s.	
Denaturation	98°C 10s.	10X
Annealing	65°C 30s	
Extension	72°C 30s	
Keep @	4°C ∞	

Final MinElute Cleanup

Follow the manufacturer's instructions, elute twice with 12µl -> will result in a total of 24 µl eluate. Use only a single column. Due to the large volume the PB/sample mixture will have to be loaded and centrifuged multiple times.

Run 1µl of the size selected and PCR product on the tapestation (together with the size selected pre-PCR product).

Elchrom Size Selection

Prepare in advance:

- autoclaved 1X TAE buffer (at least 2 litres, *i.e.*, mix 40 ml 50X TAE with 1960 ml MiliQ water)

Buffer refilling

- Plug in water tubes and pump cables. Fill the tank with ddH_2O. Start water pump and ensure that no water leaks into the running buffer.
- Fill gel tray halfway up, start the pump (this results in fewer air bubbles). With the pump running, add remaining buffer (up to 2000 ml).

Preheating

- Set temperature to 55°C. Turn on heater (black switch), water pump (green switch), and buffer pump (red switch and turning knob). Close lid.
- Turn on power unit (120 V, max. amperage).
- For the final 15-20 min, unpack gel and lay it onto the catamaran to preheat. Close lid.

Sample preparation

- Mix 5µl Elchrom loading buffer with 20µl library (load max 10µg of DNA per well to avoid overloading!).
- Prepare 0.3µl M3 marker with 1.5µl loading buffer and 3µl MiliQ water (3X).
- Take tips, pipettes, samples, tissues, and Kimwipes to the gel work bench.

Gel loading (within 2-5 min)

- When buffer is preheated and everything is ready, turn-off power unit.
- Turn buffer pump off (set to off, not power button), as well as the heater and water pump.
- Open lid and clean it with Kimwipes. Immerse gel in the gel tray, fix with catamaran.
- Remove any bubbles from the wells. Ensure gel is in the correct position.
- Load 12.5µl or 25µl of each library onto the gel (depending on gel).

- Load 5µl M3 Marker onto the gel.
- Close lid, turn on power unit. Set to 120 V, max. Amperage, and estimated running time.
- Turn on water pump and heater (but keep the buffer pump off!).
- Start the run as soon as possible. Manually start the buffer pump after ~5 min.

Staining

- Turn off power source.
- Remove gel and use nylon string (6X at least!) to get rid of the back plate.
- Fill staining tray with 50 ml of MiliQ water, 336µl 50X TAE and 10µl SYBR gold. Slide the gel gently into the tray and wrap tray in aluminium foil.
- Stain on the shaker for 30-45 min.
- Empty staining tray without removing the gel and fill with 99 ml MiliQ water and 1 ml Destaining solution.
- Destain on the shaker for 30 min.
- Remove destaining solution.

Size selection

- Slide the gel on the DarkReader. Turn on and take a picture. Use a long-bladed knife and cut out the desired size range.

Electro elution

- Prepare as many dialysis membranes as needed (approximately 3 cm longer than the gel slice) and a few extra in various sizes. Wash the membranes in 1X TAE and store them in a basin with TAE until use.
- Close one end of membrane with a clip and fill membrane with 1ml of 1X TAE.
- Put the gel piece into the membrane. Make sure the gel piece is originated in previous running direction. Close membrane with a second clip. Avoid air bubbles.
- Place gel packages in the Elchrom gel chamber. Packages should be well covered with 1X TAE buffer but not floating.
- Run overnight at 20°C (change water), set Voltage to 90V.
- Reverse polarity for 1 min to detach DNA from the membrane.
- Clean-up with the MinElute PCR purification kit. On a glass plate, pipette out the TAE buffer and dispense into a Falcon tube containing 5ml of PB buffer. Rinse the membrane with buffer to maximise recovery rate.
- Apply 750µl of the mixture on a column at a time.

Elute in 2X 12µl EB buffer each, use Lo-bind tubes.

Library QC

- Qubit
- dsDNA HS kit according to instructions

- Tapestation

HS D1000 tape according to instructions

- qPCR

The qPCR is performed with the KAPA library quantification kit

The dilution of the library (1:10,000) is performed in ddH_2O. Make three independent dilutions and pipet each dilution three times on the plate.

Stage	Temperature	Time	Reads
Holding	95°C	5:00 min	
Cycling	95°C 60°C	00:30 min 00:45 min	 X
Melt curve	95°C 60°C (ramp) 95°C 60°C	0:15 min 1°C / 1:00 min 0:30 min 0:15 min	 X

Calculate the library concentration in nM using all the above obtained data. It should give you a similar range:

Qubit:

$$concentration\ in\ nM = \frac{(concentration\ in\ ng/ul)}{(660g/mol * average\ library\ size\ bp)} * 10\text{^}6$$

Dilute the libraries to 4 or 2nM using 10nM Tris (pH 8.0) or based on the sequencing facility's instruction

ddRAD including degenerate bases for PCR duplicate detection (adapted from Tin et al., 2015)

Oligos; Adapters; Digested genomic DNA (EcoRI and MseI)

```
                          ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGAT    AATTCNNNT      TAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC
                          ||||||||||||||||||||||||||||||||||||||    ||||           ||||||||||||||||||||||||||||||
                          TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAAGCTATTAA   GNNNAAT      CTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGCGGTTANNNNTAGAGCATACGGCAGAAGACGAAC
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG 3'-->                                                                  <--3' AGCATACGGCAGAAGACGAAC
TruSeq P5/P7 read 1/2 TCTACACTCTTTCCCTACACGACGCTCTTCCGATCT                      GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
```

Final sequencing library (EcoRI and MseI)

```
TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGAAGCTATTAANNNTTACTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGXXXXXXNNNNTAGAGCATACGGCAGAAGACGAAC
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGATAATTNNNAATGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXNNNNATCTCGTATGCCGTCTTCTGCTTG
```

DNA sequence legend

Sequencing Primer reads (PCR 1 Primer, PCR 2 Primer)
Primer (P5 and P7; flowcell annealing)
Restriction site
Barcode (1 of 48)
Index (1 of 12)
Degenerate base region
Genomic DNA

“A man’s friendships are one of the best measures of his worth.”

– Charles Darwin

“Life is partly what we make it and partly what it is made by the friends we choose”

– Tennessee Williams

4. Social Bond Strength and Alliance Stability Determine Fitness in Male Dolphins

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Abstract

Variation in reproductive success, or fitness, evident between individuals has been linked to differences in physical attributes as well as social bond strength. Many studies have investigated the link between social bonds and fitness in females but much less is known of males. This is of particular interest in species where unrelated males cooperate to gain access to females. Adult male Indo-Pacific bottlenose dolphins form multi-level alliances, engaging in coordinated efforts to compete with rival alliances over females. The core social unit is the second-order alliance, comprised of 4-14 males. Nested within these second-order alliances, two to three males form first-order alliances to sequester individual females within 'consortships'. First-order alliances can vary in composition. Here, we combined genomic and behavioural data on 53 members of six second-order alliances to assess the influence of the following variables on male fitness: individual node strength (weighted degree centrality), variability in social bond strength across the second-order alliance, first-order alliance stability, home range size, relatedness, and age similarity. Social bond strength and first-order alliance stability were the only significant predictors of the number of consortships in which a male engaged, a proxy for number of paternities and thus, direct fitness. Male bottlenose dolphin fitness is therefore influenced by an individual's access to close and reliable social partners with whom to cooperate, rather than relatives, as is the case in some highly encephalised terrestrial species such as our own.

Introduction

There can be significant variation in the number of offspring produced by members of the same species (Clutton-Brock, 1988). Understanding the determinants of such differential reproductive success is at the core of evolutionary biology because of its connection to inclusive fitness. Early work focused on individual age (Komdeur, 1996), rank (Hodge et al., 2008), or size (Bercovitch, 1989; Dickerson et al., 2005), as well as habitat characteristics (Clutton-Brock, 1988). More recent studies, however, revealed that 'social bonds', *i.e.*, persisting, affiliative relationships between individuals (Cords and Thompson, 2017; Massen, 2017), can also have a strong bearing on individual fitness, affecting lifespan, offspring survival, and access to critical resources (reviewed in Massen, 2017; Seyfarth and Cheney, 2012; Silk, 2014).

Individuals living in groups can form multiple social bonds varying in number, duration and strength. Both, quantitative and qualitative aspects of social bonds have been linked to fitness. The number of strong social bonds of female chacma baboons (*Papio ursinus*), for example, positively correlated with birth rate, while their number of weak bonds predicted infant survival and longevity (McFarland et al.,

2017). Work on blue monkeys (*Cercopithecus mitis*) revealed that females forming inconsistent social bonds over multiple years had a higher risk of mortality compared to females with more persistent social bonds (Thompson and Cords, 2018).

The effects of social bonds on fitness have largely been studied in females. This bias can be attributed, at least in part, to the challenges involved in investigating the reproductive success of males, as it often requires genetic sampling to assess parentage. Moreover, male mammals tend to disperse from their natal group and also compete singly over access to females, further impeding the formation of persistent male-male bonds (Díaz-Muñoz et al., 2014). Rather than forming long-term social bonds, males primarily participate in short-term coalitions in the pursuit of a higher rank (e.g., chimpanzees *Pan troglodytes*, de Waal, 1984), or other short-term cooperative acts such as preventing territorial takeovers by other males (e.g., cheetahs *Acinonyx jubatus*, Caro, 1994; hyaenas *Hyaena hyaena*, Wagner et al., 2008). As a consequence, differential reproductive success among males has predominantly been investigated in terms of fighting ability or strength (Clutton-Brock et al., 1979; Lappin and Husak, 2005), home range size (Fisher and Lara, 1999), or social status (Cowlishaw and Dunbar, 1991; Le Boeuf, 2015), but rarely with regards to the quantity and quality of social bonds.

Male-male social bonds have traditionally been explored using a framework of kin selection, invoking both direct and indirect fitness benefits (Buchan et al., 2003; Hamilton, 1964; Pope, 1990). Indeed, as expected under kin selection, social bonds among males are often biased towards relatives (Smith, 2014). However, social bonds among unrelated males have been shown to influence reproductive success in lekking birds, where males cooperate in courtship displays (McDonald and Potts, 1994). Social bond strength among male Assamese macaques (*Macaca assamensis*) also predicted coalition formation, which positively influenced future dominance and thus increased reproductive success (Schülke et al., 2010).

Male bottlenose dolphins (*Tursiops aduncus*) in Shark Bay form multi-level alliances to sequester oestrus females (Connor and Krützen, 2015). 'Second-order alliances', consisting of 4-14 males, are the core social unit that can last for decades (Connor and Krützen, 2015). They reside in a continuous mosaic of overlapping home ranges but, in contrast to other alliance-forming species, such as chimpanzees, male dolphins do not engage in territorial defence (Randić et al., 2012). Second-order alliances are defined both by their social bond strength and their functional behaviour, cooperating in the herding and defence of females (Connor and Krützen, 2015). However, social bond strength does vary within second-order alliances, with some males forming strong bonds with only a subset of their allies, whilst other males have more uniformly distributed social bonds with allies (Connor et al., 2001).

Within second-order alliances, two to three males form 'first-order alliances' to herd and mate with fertile females in 'consortships' (Connor et al., 1996). First-order alliances may vary in composition over different consortships, with some males almost always consorting with the same allies (high first-order alliance stability), whilst others do not exhibit such distinct partnership preferences (Connor and Krützen, 2015). Previous research has shown that males with more stable first-order alliance partners spend a significantly higher proportion of days consorting females compared to males that do so with a changing subset of partners (Connor and Krützen, 2015).

Alliance formation is critical for male reproductive success as non-allied males sire few, if any, offspring (Krützen et al., 2004a). We have previously illustrated that first-order partner choice and second-order alliance membership are influenced by social bond strength and not kinship, with second-order alliances consisting largely of similarly-aged males (Chapter 3). However, we do not yet know the extent to which social and non-social factors influence reproductive success in male dolphins. The aims of this study were thus twofold: (*i*) to examine whether number of consortships predict reproductive success in male dolphins; and (*ii*) to investigate the degree to which social and non-social factors, such as occurrence and variation in social bond strength, first-order partner stability, age similarity, kinship, and individual home range size, influence number of consortships among second-order alliance members. We predict that: (*i)* a male's paternity success will be reflected in his consortship success, because males gain access to females within consortships and; (*ii)* a male's consortship success will largely be influenced by social factors, because males invest in social relationships from an early age (Krzyszczyk et al., 2017) and the low availability of kin impedes alliance formation based on kin selection (Chapter 3). Our study provides novel insights into how social and non-social variables influence reproductive success in a complex, multi-level society where males cooperate over access to females.

Materials and Methods

Study site and behavioural data collection

This study is based on behavioural and genetic data collected from the dolphin population in the eastern gulf of Shark Bay, Western Australia. Behavioural data in the form of surveys and large scale consortship data have been collected on a near-annual basis since 1984 and 2001, respectively (Smolker et al., 1992). Genetic sampling, using a biopsy system specifically designed for small cetaceans, has been an ongoing effort since 1994 (Connor and Krützen, 2015; Krützen et al., 2002).

Here, we accessed survey data of group composition, based on the 10m chain rule (Smolker et al., 1992), GPS locations, and consortship data, based on established criteria (details in the supporting information). We restricted survey data to the period 2001-2018, from which we have both genetic and behavioural data available from multiple male alliances.

Association between number of consortships and paternities

We examined whether a male's number of consortships was linked to his reproductive success. To that end, we assigned paternities to individual adult males by running a paternity analysis based on genetic microsatellite data in *CERVUS* v3.0.7 (Kalinowski et al., 2007) at the 95% confidence level (more details in the supporting information).

We limited consortship data from 2001 until 2012, one year prior to the birth of the youngest calf with an assigned father, taking into account the one year gestation period (Schroeder, 1990). To ensure that the consortships and paternities occurred in the same time period, we estimated paternities for 104 calves born between 2002 and 2013.

To investigate the predictive power of a males' number of consortships on his number of paternities, we built a Poisson zero-inflated generalised linear mixed model using the R package *glmmTMB* (Brooks et al., 2017) in R v3.6.2. This allowed us to account for the low number of males to which we were able to assign paternities (Table 4.1, Paternity Model). Our model included an unprecedented dataset using long-term consortship and paternity data of allied adult males for which genetic data was available (N = 116). To test whether the number of consortships predicted achieved paternities, the Paternity Model included number of paternities per male as the dependent variable, his number of consortships as the predictor variable, and second-order alliance membership as a random factor. Alliance membership was included as random factor because behavioural sampling effort varied over different second-order alliances but was consistent within alliances. Summary statistics, including the

P-values for statistical significance from Wald Z-tests, were obtained using the *car* package (Fox and Weisberg, 2019).

Focal males to investigate variation in consortship success

To identify which social and non-social factors influenced consortship success between second-order alliance members, we identified six second-order alliances consisting of 58 well-known males. By using consortships instead of paternities, we could maximise the number of males in this analysis. The analysis was carried out on 53 males, referred to as 'focal males' hereafter, due to missing data for five males (details on this restriction in the supporting information). We confirmed second-order alliance membership between 2001 and 2018 as described in Chapter 3. Each member of these alliances had been surveyed on average 149.5 ± 95.3 (min = 37, max = 389) times between 2001 and 2018, and participated in 26.3 ± 21.8 (min = 2, max = 74) consortships. Details on alliance membership of the focal males are provided in the supporting information (Table S4.1).

Social variables to investigate variation in consortship success

To infer how social factors were related to an individual's number of consortships, we calculated individual first-order alliance stability, social bond strength to second-order alliance members, and the coefficient of variation thereof. First-order alliance stability (1 - (# different first-order alliances/# consortships)) was calculated for all focal males that engaged in three or more consortships (54 out of 58) as described in (Connor et al., 2001).

Social bond strength to second-order alliance members was inferred by calculating each focal male's node strength, also referred to as weighted degree centrality (Barrat et al., 2004), and measured via patterns of associations. Associations were quantified using the simple ratio index (SRI) based on group composition during the first five minutes of surveys. We calculated SRI values in the R environment using *asnipe* (Farine, 2013), and obtained node strength by summing up an individual's SRI values to his second-order alliance members. Node strength does not contain information about variability in bond strength, e.g., if a male has equally strong bonds to all of his alliance partners or has preferred and avoided partners. Thus, we also calculated each focal male's coefficient of variation (CVs) based on their SRI values to their second-order alliance members. Smaller CVs indicate that individuals associate more evenly with their second-order alliance members, while higher CVs indicate more heterogeneous associations with second-order allies, *i.e.,* preferred and avoided second-order alliance members.

Non-social variables to investigate variation in consortship success

To infer the influence of non-social variables on number of consortships, we estimated pairwise relatedness using the triadic likelihood estimator (TrioML) implemented in *Coancestry* (Wang, 2011). For this, we used 9,991 single nucleotide polymorphisms (SNPs) produced from a double digest restriction-site associated (ddRAD) DNA sequencing approach (described in Chapter 3). We also estimated age difference to second-order alliance partners (Gerber et al., 2020), and individual home range size of the focal males between 2001 and 2018, *i.e.*, the years for which consortship data were available. Home range sizes were obtained as described in Wild et al. (2019a). However, instead of dyadic home range overlaps between individuals, we calculated individual home range sizes using the 95% kernel of the utilisation distribution in km^2, as included in the R package *adehabitatHR* (Calenge, 2011).

Statistical analyses to identify determinants of consortship success

To assess the extent to which social and non-social variables determined consortship success of individual males, we built a Poisson generalised linear mixed model (GLMM) in R using the *lme4* package (Bates et al., 2015). In this model, we entered a focal male's number of consortships as a dependent variable to infer consortship success and first-order alliance stability, node strength, CV of social bond strength (SRI) for the second-order alliance, average age difference to second-order alliance members, average relatedness to second-order alliance members, and individual home range size as explanatory variables (Table 4.1, Consortship Model). As in the Paternity Model, we included second-order alliance membership as random factor. Not all males were members of an alliance in all years studied but instead, joined at a later point in time or disappeared, so we included each male's number of years present in a second-order alliance as an offset term.

Since the Consortship Model contained multiple variables, we employed an information-theory approach to infer the best model. For this, we calculated a global model containing all variables, as well as models containing all possible subsets of the explanatory variables. We then ranked these models based on their Akaike's information Criterion (AIC) values corrected for small sample size (AICc). We included all models within a cumulative Akaike weight of ≥ 0.95 from the top model, thereby creating a '95% confidence set' (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). Model selections were carried out in the *MuMIn* package (Barton and Barton, 2018). To ensure absence of multicollinearity among variables, we calculated the variance inflation factor (VIF) using the *car* package (Fox and Weisberg, 2019).

Table 4.1. Overview of the Paternity and Consortship Model.

Paternity Model	$\#Paternities_{Individual} \sim \#Consortships_{Individual} + (1|Alliance)$
Consortship Model	$\#Consortships_{Individual} \sim$ Alliance Stability + Strength + CV + Δage + r + HR + offset(log(Years in Alliance)) + (1|Alliance)

$\#Paternities_{Individual}$ = number of paternities per male, $\#Consortships_{Individual}$ = number of consortships per male, Alliance = second-order alliance, Alliance stability = first-order alliance stability, Strength = node strength, CV = coefficient of variation of SRIs, Δage = age difference in years, HR = individual home range size, Years in Alliance = number of years a male was a member of a specific second-order alliance.

Results

Association between number of consortships and paternities

Of 104 calves born between 2002 and 2013, we successfully assigned 51 to 116 candidate fathers, which were members of 13 second-order alliances. The number of individual consortships engaged in by candidate males ranged from 0 to 51 (mean = 16.12 ± 11.68), while paternities ranged from 0 to 5 (mean = 0.44 ± 0.83). Consortships were thus revealed as a suitable proxy for reproductive success, as the number of consortships in which a male engaged significantly predicted the number of paternities he achieved (N = 116, *P* = 0.025, Table 4.2: Paternity Model and Fig. 4.1).

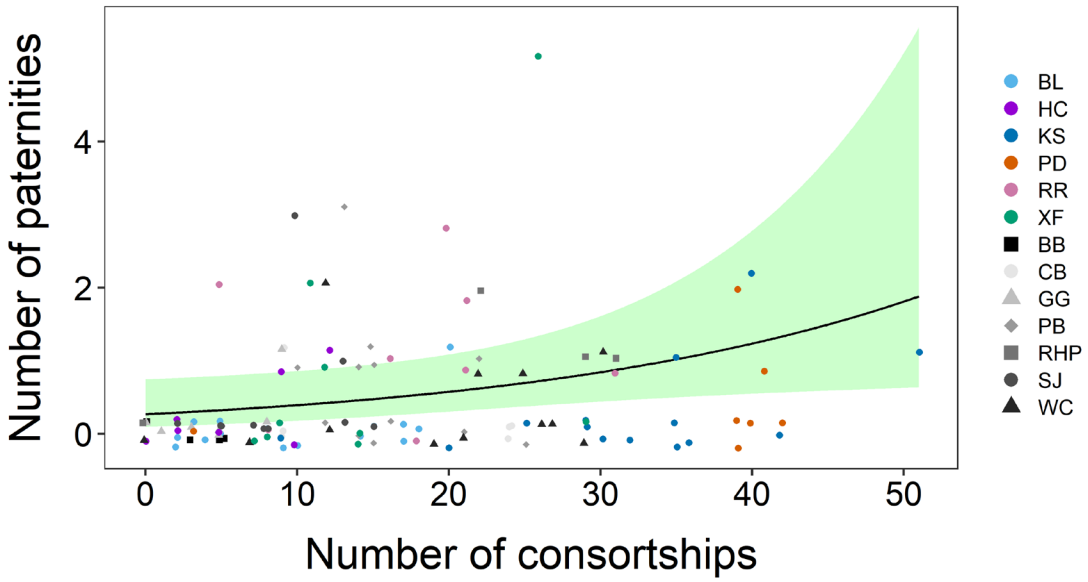


Figure 4.1. Predictive effect of consortships on paternities. The number of consortships in which a male engaged significantly predicted the number of paternities he scored (zero-inflated GLMM, *P* = 0.025, conditional R^2 = 0.497). The black line represents the predictor effect of paternities on consortships with the confidence interval in green. The raw values of the individual males (N = 116) are plotted on the graph. Coloured points indicate members of the six focal alliances. Various shapes in shades of grey represent members of other well-known second-order alliances.

Determinants of consortship success between second-order alliance members

Of all social (first-order alliance stability, node strength, CV of social bond strength) and non-social variables (relatedness, age difference, home range size) investigated, only first-order alliance stability (N = 53, *P* = 0.001, Fig. 4.2) and node strength (*P* = 0.020, Fig. 4.3), were significant predictors of a male's number of consortships (averaged model results are given in Table 4.2, detailed summary statistics on the 95% confidence set in Table S4.2). This finding indicates that those males that consorted more frequently with the same subset of first-order alliance partners and with stronger overall bonds to their second-order alliance members achieved the highest number of consortships.

Table 4.2. Detailed results from the Paternity Model.

	B	***S.E.***	***z-value***	***P-value***
Paternity Model				
Intercept	-1.314	0.521	-2.523	**< 0.012**
Number of consortships	0.038	0.017	2.240	**0.025**
Consortship Model				
Intercept	1.247	1.122	1.112	0.266
first-order alliance stability	0.640	0.273	2.329	**0.020**
Node strength	0.622	0.194	3.184	**0.001**
Coefficient of variation	-0.093	0.147	0.630	0.529
Age difference	-0.020	0.058	0.333	0.739
Relatedness	-0.293	0.190	1.527	0.127
Home range size	0.001	0.193	0.007	0.995

Detailed results from the Paternity Model (N = 116) investigating the correlation between number of consortships and paternities and the Consortship Model (N = 53) assessing the influence of different variables on an individual's number of consortships. Estimates were averaged across the 95% confidence set and results are reported for the full average (model weights are provided in Table S4.2).

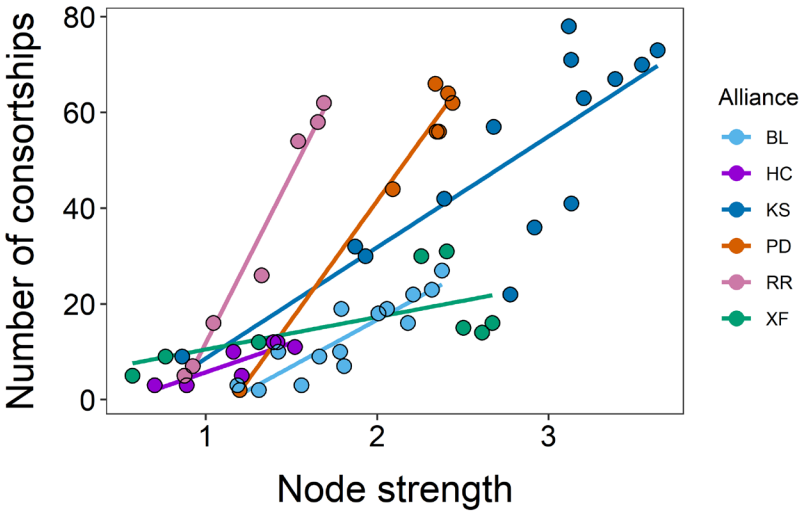


Figure 4.2. A male's number of consortships positively correlated with his node strength (GLMM, *P* = 0.001). Each dot represents a focal male's number of consortships and his node strength. Data are colour-coded by alliance membership and linear regressions through all males belonging to the same second-order alliance are shown.

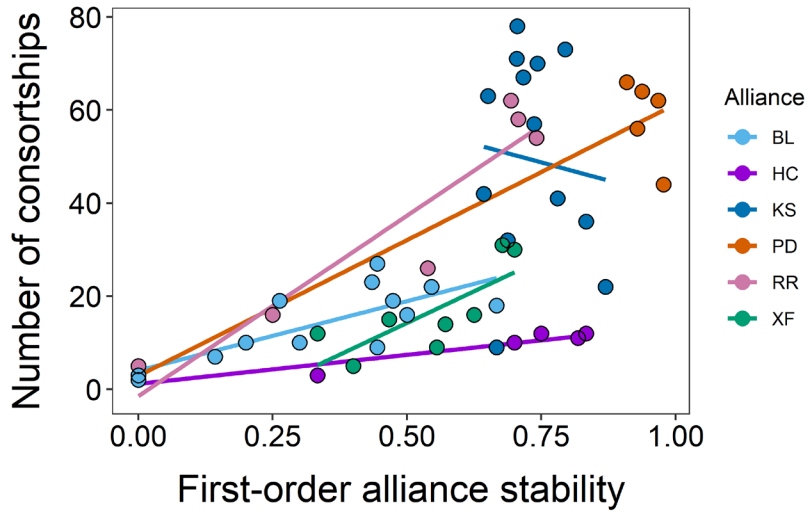


Figure 4.3. A male's number of consortships positively correlated with his first-order alliance stability (GLMM, *P* = 0.020). Dots represent focal males and contain their respective number of consortships and first-order alliance stability. Data are colour-coded by alliance membership and linear regressions through all males belonging to the same second-order alliance are shown.

Discussion

Consortships provide reliable information on male fitness in the Shark Bay dolphin population, as we show here that a male's number of paternities was predicted by the number of consortships in which he engaged. Similar patterns, where male mating behaviour is correlated with reproductive success, have been found in other mammals (Amos et al., 1993; Coltman et al., 1999; Mooring and Penedo, 2014; Pemberton et al., 1992; Say et al., 2003). The relative reproductive success of males in our system can therefore be inferred via behavioural measures, enabling us to investigate how social and non-social variables influenced reproductive success among allied male dolphins.

Our findings confirm the previously reported relationship between first-order alliance stability and consortship rate, *i.e*, the number of days a male was seen consorting, divided by the total days that male was observed (Connor et al., 2001; Connor and Krützen, 2015). In this study, however, we found that node strength, the sum of a male's social bond strengths to his second-order alliance members, is also a significant predictor of a male's number of consortships. Interestingly, this suggests that a male's success in consorting females, and, by proxy, his paternity success, is not simply related to the stability of his first-order alliance, but also to the full extent and magnitude of his bonds with other males in his second-order alliance. The coefficient of variation on social bond strength had no effect on a male's success consorting females, showing that this social network effect occurs independently of whether males have more homogeneous social relationships to their second-order allies or pronounced preferences and avoidances.

Social bonds govern the life of male dolphins from an early age in Shark Bay, with male calves and juveniles investing more time in social activities than females of the same age (Krzyszczyk et al., 2017). Furthermore, social bonds formed during adolescence persist well into adulthood (Gerber et al., 2020). We build upon these previous studies by providing new evidence that social bonds among male dolphins affect their reproductive success and are, therefore, directly linked to fitness.

Adaptive benefits of social bonds include lower exposure to aggression from conspecifics (Cameron et al., 2009), lower risk of predation (Micheletta et al., 2012), reduced levels of stress (Young et al., 2014a), and better immune response and health (Capitanio et al., 1998). While all these effects might contribute to a male dolphin's lifetime reproductive success, the positive effect of social bonds on cooperation is probably most pronounced. Social bonds can facilitate cooperation (Asakawa-Haas et al., 2016; Samuni et al., 2018) and, hence, an individual's access to crucial resources such as food or mates (Thompson, 2019). With cooperation frequently being biased towards close social partners, individuals with a larger number of social bonds might be in a better position to harness the benefits

of cooperation. Indeed, we found that a male's number of consortships increases with his node strength, suggesting that his level of social integration within the second-order alliance influences his fitness. Males with higher node strengths might be chosen more often as first-order alliance partners since they are, overall, more closely bonded to their second-order alliance members. Furthermore, such well-integrated males might cope better with partner loss than those with fewer close partners, as observed in bats (Carter et al., 2017). Similar to other species (Gilby et al., 2013; Loretto et al., 2012; Schülke et al., 2010), social bond strength of male dolphins might predict coalitionary aggression and, thereby, well-connected males might be better positioned to steal or defend a female compared to males with weaker social bonds.

Males consorting more frequently with the same first-order alliance partners, *i.e.*, with higher first-order alliance stability, attained a higher number of consortships. Consorting repeatedly with one another might improve their ability to coordinate. As consortships occur in a three-dimensional habitat and can last several weeks (Connor et al., 1996; Connor et al., 1992), familiar and well-coordinated males might be more capable of defending their female consorts from attacks or preventing her from escaping. In addition to motor coordination, allies engage in remarkable levels of acoustic coordination during consortships, including synchronised production of threat vocalisations (Moore et al., 2020). Familiarity enhances coordination between individuals in other species living in three dimensional habitats, including some birds (Prior et al., 2019), freshwater fishes (Riley et al., 2019), and juvenile sharks (Keller et al., 2017). Male dolphins in Shark Bay surface in tight synchrony more often with first- than second-order allies, although synchrony with second-order allies occurred more during socialising with consorted females, possibly serving to reduce tension (Connor et al., 2006). Synchronous behaviours have been shown to increase bond strength in multiple species, potentially through the release of endorphins (Tarr et al., 2016) and oxytocin (Jiang and Platt, 2018; Spengler et al., 2017), which promotes bonding and facilitates cooperative relationships (e.g., chimpanzees, Crockford et al., 2013; humans, de Dreu et al., 2010; dogs *Canis familiaris*, Romero et al., 2014). Since oxytocin is universal to mammals, it could well be that these effects are shared in dolphins. Thus, bond strength might increase cooperation within consortships or, *vice versa*, repeated consortships might increase social bond strength.

Males preferentially form first-order alliances with those they are closely bonded with, rather than close relatives or members of a similar age (Chapter 3). Our results support and, indeed, expand upon these findings by demonstrating that relatedness and age difference to second-order alliance members did not influence a male's number of consortships, while social factors did. We detected no predictive role of home range size, the third non-social variable investigated, for a male's number of consortships. This is surprising because larger home ranges are expected to allow access to a higher

number of fertile females. Correlations between male home range size and reproductive success have been documented in solitary carnivores (Kovach and Powell, 2003; Sandell, 1989). However, male bottlenose dolphins need to cooperate with others to consort females (Krützen et al., 2004a). Since the home ranges of multiple second-order alliances overlap, they are expected to compete over the same females. The ability to defend or steal females from others via cooperation therefore appears to have a more pronounced effect on fitness than home range size.

Here, we assessed multiple aspects of social bonds and show that their adaptive benefits outweigh the effects of other, more traditionally studied, attributes. Selection not only acts upon phenotypic but also behavioural traits. Group-living entails social interactions, thereby creating near endless opportunities to cooperate or compete. Selection is thus expected to favour social strategies that benefit individuals. This is particularly true for long-lived species, in which individuals are capable of individual recognition and have the opportunity to form long-lasting friendships. Further, selection might act upon cognitive abilities that allow an individual to manipulate its social environment through, for example, cooperation with specific individuals. Humans have a tendency to form strong social bonds outside of kin, which are connected to fitness (Holt-Lunstad et al., 2010), and research on non-human primates has indicated that the evolutionary roots of non-kin social bonds influencing fitness can be observed outside of our direct lineage (Ostner and Schülke, 2014; Silk, 2007b). Our long-term dolphin study provides additional evidence that strong social bonds among unrelated males correlate with fitness in a highly encephalised, non-primate species with a vastly different evolutionary history.

Acknowledgements

We thank Monkey Mia Dolphin Resort, Monkey Mia Wildsights, and the DBCA's Shark Bay Rangers for their continued support and assistance. We also thank all field assistants for their help during this study and the Genetic Diversity Centre (GDC), ETH Zurich, for their support in data production.

Supporting Information

Paternity analysis

Paternity assignment was based on 27 polymorphic microsatellite loci (E12, D22, D8, F10, EV37, MK3, MK5, MK6, MK8, MK9, KWM12, Tur4_66, Tur4_80, Tur4_87, Tur4_91, Tur4_98, Tur4_105, Tur4_108, Tur4_111, Tur4_117, Tur4_128, Tur4_132, Tur4_138, Tur4_141, Tur4_142, Tur4_153, Tur4_162, (Hoelzel et al., 1998; Krützen et al., 2001; Nater et al., 2009) amplified and scored according to established protocols, as described in (Allen et al., 2016). We used CERVUS v3.0.7 (Kalinowski et al., 2007) for paternity assignment. After removal of duplicates and individuals with fewer than 15 loci genotyped, we calculated population-specific allele frequencies over all individuals sampled within the study site (N = 650) and checked that all markers were in Hardy-Weinberg equilibrium. We ran 10,000 simulated parentage analyses to obtain the critical delta value of 5.68 for 95% confidence with the father alone, and 2.31 in cases where the mother was known. Mothers were identified behaviourally, *i.e.*, from multiple sightings of a female with a dependent calf. We set the number of candidate fathers to 430, regarded a conservative overestimate of the male dolphin population size in eastern Shark Bay (Krützen et al., 2004a). Of these, we assumed a sampling proportion of 1:3. The proportion of loci typed was based on the genotyped data (0.968) and the proportion of mistyped loci was set to 0.01. Due to the inherent social structure, with male and female philopatry (Krützen et al., 2004b), we set the proportion of related candidate males to 0.1 at a relatedness of 0.104, as previously established for paternity assignments within the same population (Krützen et al., 2004a). Using these parameters, we assigned a total of 51calves to 34 fathers.

Target alliances

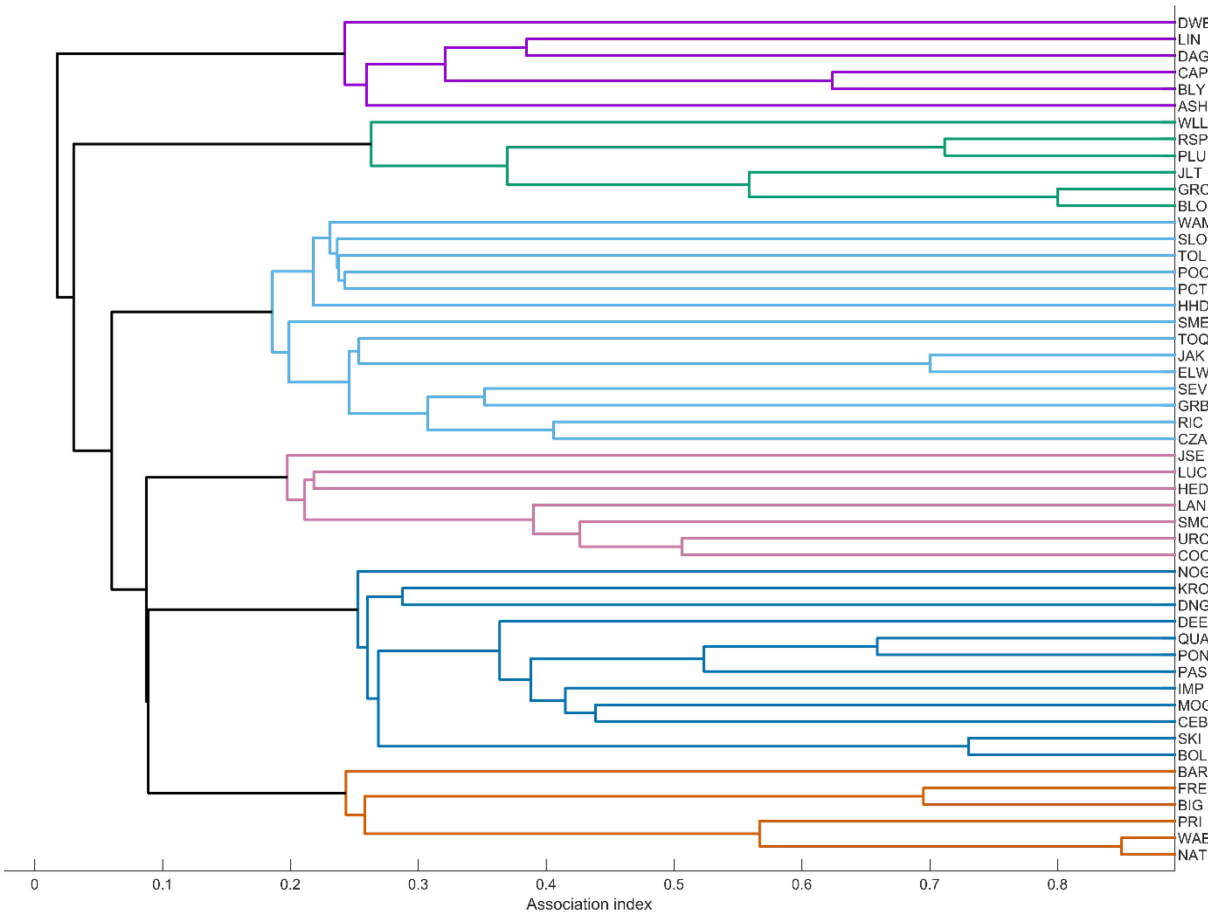


Figure S4.1. Dendrogram of this study's target alliances, as identified with the fast and greedy (Clauset et al., 2004) community detection algorithm based on survey data. The 58 focal males were distributed over six second-order alliances, each consisting of 7-14 individuals. Males BAK, DWE, HHD and WAM were excluded from the Consortship Model because they participated in fewer than three consortships. Male JAA was excluded because his year of birth and, thus, his age difference to alliance partners could not be determined.

Table S4.1. Overview of the six second-order alliances.

Alliance	ID	Consortships	Paternities	Alliance	ID	Consortships	Paternities
BL	***CZA***	20	0	KS	***NOG***	31	0
BL	***ELW***	19	0	KS	***PAS***	58	0
BL	***GRB***	11	0	KS	***PON***	67	1
BL	HHD	2	0	KS	***QUA***	61	0
BL	***JAK***	18	0	KS	***SKI***	35	0
BL	***PCT***	9	0	KS	***TER***	9	0
BL	***POO***	9	0	PD	***BAR***	3	0
BL	***RIC***	23	1	PD	***BIG***	66	0
BL	***SEV***	14	0	PD	***FRE***	64	1
BL	***SLO***	4	0	PD	***NAT***	51	0
BL	***SME***	6	0	PD	***PRI***	39	2
BL	***TOL***	5	0	PD	***RID***	63	0
BL	***TOQ***	16	0	PD	***WAB***	51	0
BL	WAM	2	0	RR	***COO***	50	2
HC	***ASH***	5	0	RR	***HED***	16	1
HC	BAK	2	0	RR	***JSE***	8	0
HC	***BLY***	10	0	RR	***LAN***	26	3
HC	***CAP***	10	0	RR	***LUC***	5	2
HC	***DAG***	10	1	RR	***SMO***	46	0
HC	DWE	2	0	RR	***URC***	49	1
HC	***LIN***	12	1	XF	***BLO***	14	0
KS	***BOL***	40	2	XF	***EXF***	8	0
KS	***CEB***	65	0	XF	***FAR***	7	0
KS	***DEE***	74	1	XF	***GRC***	12	1
KS	***DNG***	42	0	XF	JAA	9	0
KS	***IMP***	52	0	XF	***JLT***	14	0
KS	***KRO***	32	0	XF	***PLU***	29	0
KS	***MID***	20	0	XF	***RSP***	26	5
KS	***MOG***	66	0	XF	***WLL***	11	2

Overview of the six second-order alliances with genetic data available and each individual's number of consortships. The males printed in bold italics denote the 53 focal males. Males HHD, WAM, BAK, and DWE engaged in fewer than three consortships, while JAA was excluded due to missing age information.

Table S4.2. Summary statistics on the 95% confidence set model

Terms	***Log likelihood***	***AICc***	***ΔAICc***	***Weight***
13567	-187.00	387.82	0	0.19
34567	-185.77	388.02	0.2	0.17
1367	-188.55	388.38	0.56	0.14
234567	-185.13	389.54	1.72	0.08
134567	-186.84	390.17	2.35	0.06
123567	-186.84	390.17	2.35	0.06
13467	-188.26	390.34	2.52	0.05
1567	-189.55	390.37	2.55	0.05
12367	-188.36	390.54	2.73	0.05
167	-191.07	390.98	3.16	0.04
12567	-189.00	391.83	4.01	0.03
1267	-190.84	392.24	4.42	0.02
123467	-188.09	392.67	4.85	0.02
1234567	-186.70	392.67	4.85	0.02
3467	-189.45	392.73	4.91	0.02

Term codes: 1 = offset (log(Years spent in Alliance)); 2 = Average age difference to alliance partners; 3 = Average relatedness to alliance partners; 4 = CV of SRI; 5 = Home range size; 6 = first-order alliance stability; 7 = Node strength

Variance Inflation Factors

First-order alliance stability	1.792
Average age difference	1.138
Average relatedness	1.077
Home range size	1.163
Node strength	1.555
CV	1.683

Shark bay ethogram – consortship criteria

Consortships are coercively maintained associations between two to three males and one female. Consortships are scored as YES (Y), NO (N), or PROBABLE (P) for both the male subgroup and suspected female consort. Scoring the consortship as a YES for both males & female consort requires satisfaction of the following criteria:

A) The subgroup of two to three and one female is observed with >10m separation between any other individual or group, AND

B) ONE or more of the following six events are observed:

1. **1hr:** Consortship subgroup is observed for at least one hour. At least one male from the consortship subgroup is within 10m of the suspected female throughout the one-hour observation period.
2. **Capture:** The female is captured by an alliance of males.
3. **Bolt:** Female attempting to escape by rapidly swimming ('bolting') from an alliance of males
4. **Pops:** At least one of the males producing a vocal threat called 'pops' that induces the female to remain close (Connor& Smolker, 1996)
5. **Directed Aggression:** At least one of the males directing physical threats or aggression toward the female. Aggressive behaviour described in the Shark Bay Dolphin Research Ethogram.
6. **Theft:** teams of two alliances attempting to take a female from another alliance (a 'theft'; Connor et al., 1992a,b) ** In this case, the consortship is scored as a "Y" for loosing males, winning males, and female.

If A observed but B is not observed, the consortship should be scored as a "P" if:

> 10m separation, one female, > 30 min but < 1hr observation ("P" for males & female)

“Consider them both, the sea and the land; and do you not find a strange analogy to something in yourself?”

– Herman Melville

5. General Discussion

With this PhD thesis, I sought to piece together the puzzle of male alliance formation in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) through a combination of long-term behavioural and genomic data. In summary, the work presented here suggests that kinship does neither influence the formation and maintenance of alliance bonds (Chapters 2 and 3), nor does it predict paternity success (Chapter 4). Rather, my results indicate that close social bonds, in particular those that were formed early in life and during adulthood to other second-order alliances members, govern the lives of male bottlenose dolphins. In the general discussion I attempt to integrate my findings into a larger framework. In the first section, I evaluate some ultimate mechanisms that might underlie male cooperation in bottlenose dolphins. This is followed by reviewing partner choice and ontogenetic aspects of male cooperation in the absence of kin selection in other species and how they compare to my findings. In the third section I discuss why there might be complex non-kin alliances in Shark Bay and how this compares to other bottlenose dolphin populations, while the fourth is dedicated to methodological issues pertaining to relatedness estimation and the identification of social bonds in dolphins. Each section contains suggestions for future research, but I also provide additional ideas in a fifth and final section.

Male alliance formation in dolphins and its ultimate mechanisms

Based on the results presented in Chapters 2-4, it seems highly unlikely that male alliances in Shark Bay dolphins are based on kin selection. Other ultimate mechanisms explaining cooperation among non-kin include by-product mutualism (Connor, 1995; West-Eberhard, 1975), market effects (Noë and Hammerstein, 1994, 1995), pseudo-reciprocity (Connor, 1986), and reciprocity (Trivers, 1971b).Carefully designed experimental studies are needed to distinguish between these mechanisms. Such experimental studies were, for example, carried out in vampire bats (*Desmodus rotundus*) where food sharing turned out to be best explained by reciprocity instead of alternative explanations such as harassment or kin selection (Carter and Wilkinson, 2013). Although a recent study found that that the same vampire bats which cooperated under controlled laboratory conditions kept cooperating in the wild (Ripperger et al., 2019), social and ecological variables cannot be controlled for in natural populations. Thus, studies conducted in captivity do not necessarily allow to draw conclusions about the mechanisms in place in the wild. While the inference of ultimate mechanisms of cooperation in wild populations is difficult, it is not impossible. Experimental setups and detailed behavioural observations on wild populations revealed that cooperation in male lions (*Panthera leo*) can be explained by mutualism (Grinnell et al., 1995). Cooperative behaviours in vervet monkeys (*Chlorocebus pygerythrus*, Borgeaud and Bshary, 2015), chimpanzees (*Pan troglodytes*, Gomes and Boesch, 2011; Newton-Fisher and Lee, 2011), and pied flycatchers (*Ficedula hypoleuca*, Krams et al., 2008) could be attributed to market effects and reciprocity. Owed to the various definitions of reciprocity and its similarity to market effects though, market effects and reciprocity are difficult to distinguish. Identifying the ultimate mechanisms underlying male cooperation in dolphins or other marine mammals might still be a far-reaching goal in the foreseeable future. Behavioural data collection on cetaceans is primarily conducted from boats. Therefore, individual behaviours and social interactions are missed when individuals are not at the surface. Notwithstanding, the insights gained into the proximate mechanisms of dolphin male alliances in the frame of this thesis create space to setting up predictions on the ultimate mechanism underlying cooperative alliances in bottlenose dolphins.

By-product mutualism

Cooperative acts that are based on by-product mutualism are maintained by selfish behaviours that coincidentally benefit others (Connor, 1995; West-Eberhard, 1975). In some occasions, these benefits are increased by coordinated action (Connor, 1995). Male dolphins have an inherent and hence, self-serving interest to consort females because it increases their reproductive success. Compared to a

single male, multiple males are better capable of preventing a female from escaping and being stolen by other males. Coordinated action of multiple males within consortships might therefore increase a male's consortship success. Cooperation with other males in consortships, therefore, appears to fit the assumptions of by-product mutualism. In my opinion, however, alliance formation in bottlenose dolphins cannot be attributed solely to by-product mutualism, because it does not involve an investment but instead, results from individuals pursuing their own interests without incurring costs. In the case of cooperative alliances in bottlenose dolphins, males invest into their second-order alliance members already from an early age onward by forging social bonds to their future allies during adolescence (Chapters 2 and 3). On the level of first-order alliances, males invest into their preferred first-order alliance partners by primarily associating with them outside of the mating season and in contexts which are unrelated to mating (Chapter 3).

To rigorously test whether by-product mutualism can indeed be excluded as the ultimate mechanism that underlies cooperation in male alliances, we need data on males that lack such an investment. This could for example be males whose associates disappeared when transitioning from adolescence to adulthood, lost their alliance partners in adulthood, or associated less with their second-order alliance members outside of the mating season. If reproductive success in these males was lower, by-product mutualism could be excluded.

Pseudo-reciprocity

Allied males jointly defend or steal females from other alliances, marking a considerable investment. This mutual dependency requiring everyone's investment fits with the assumptions of pseudo-reciprocity. However, in the case of pseudo-reciprocity all individuals gain benefits after an initial investment. In instances where second-order alliance members help a subset of their second-order members to steal or defend a female, they might not gain access to said female but only experience costs. Such costly responses are usually indicators of reciprocity or a biological market. Nevertheless, it remains to be investigated whether males coming to aid indeed only experience costs or if they are rewarded with access to the female they helped to defend or steal.

Biological market

Biological market theory understands cooperation as an exchange of services or resources which is influenced by supply and demand (Noë and Hammerstein, 1994, 1995). Depending on whom an individual chooses to cooperate with, different costs and benefits are experienced, making partner choice a crucial element of biological markets (Noë and Hammerstein, 1994). Because of kin selection, relatives are the most valuable partners (Hamilton, 1964). However, my results from Chapters 2-4

suggest that social bonds rather than genetic ties predict cooperation in Shark Bay dolphins. Therefore, familiarity appears to be of higher value than relatedness.

To understand the market forces acting on male alliance formation, future studies will need to assess which traits promote the initial formation of social bonds in order to identify which traits are valued in social partners. Owing to the intensity of inter-alliance fights, males might prefer physically strong alliance members. If this was true, adolescent males would aim to forge social bonds with other males that are physically strong. Similarly, within second-order alliances, the strongest adult males would associate in the non-mating season and form first-order alliances during the mating season. In line with this, my finding that males with the highest first-order alliance stability obtained the most consortships (Chapter 4) could be caused by the strongest males repeatedly consorting together and not only by their familiarity with each other's behavioural responses. Whether this is the case could be assessed by measuring body length, or better circumference, as a proxy for strength. Body size estimates can be gained via stereo-laser photogrammetry (van Aswegen et al., 2019) or unmanned aerial vehicles (UAVs) (Christiansen et al., 2019). While the latter has only been verified in whales which are significantly larger compared to dolphins, stereo-laser photogrammetry has been successfully tested in the Shark Bay dolphin population (van Aswegen et al., 2019).

Reciprocity

Alliance members are familiar with each other from an early age onward (Chapters 2 and 3) and associate year-round, providing them with numerous opportunities for reciprocal exchanges. Furthermore, allies frequently engage in risky behaviours creating the possibility for costly responses. Indeed, it has been argued that dolphins are potentially one of the strongest cases to establish reciprocity outside of humans (Connor, 2010). On the level of second-order alliances, helping alliance members who are attacked by others without getting access to their consort, but being helped when in need, is an example of such a reciprocal exchange (Connor, 2010). Repeatedly consorting with the same males allows males to reciprocate behaviours on the level of first-order alliances. Among first-order alliance partners, guarding the female while allowing another male to forage, could be based on reciprocity if males take turns guarding the female (Connor, 2010).

Overall, male alliance formation in bottlenose dolphins appears to be best explained by reciprocity or market effects (Fig. 5.1). Cooperation within alliances is highly varied and occurs in various contexts. Thus, not all behaviours might follow the same rules and as a consequence, underlie various mechanisms. Nevertheless, having excluded kin selection as the ultimate mechanism for male alliance formation on the first- and second-order alliance level, new avenues will be opened up by investigating male cooperation on the ultimate level in male bottlenose dolphins.

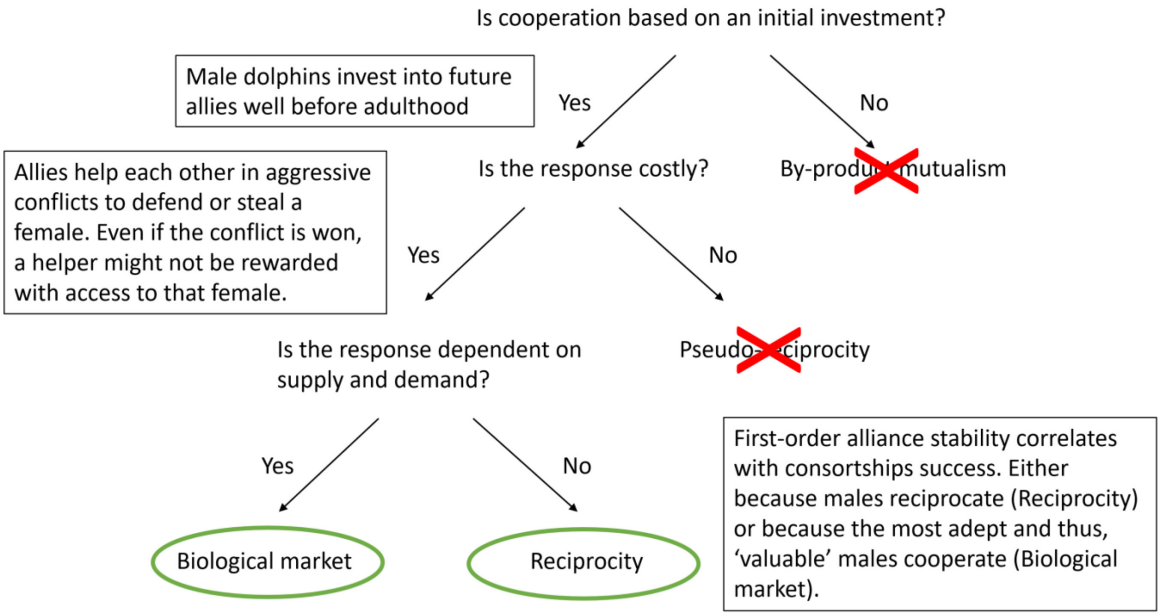


Figure 5.1. Based on the flow chart introduced in Chapter 1, cooperation in male bottlenose dolphins seems most likely to be based on reciprocity or adhere to the rules of a biological market (modified and expanded from Bergmüller et al., 2007).

Reproductive success, partner choice and ontogenetic aspects of cooperation in unrelated males

Stable cooperative relationships among unrelated males have been found in multiple species including lekking birds (du Val, 2007; McDonald and Potts, 1994; Ryder et al., 2011), lions (Packer et al., 1991), chimpanzees (Langergraber et al., 2007; Mitani et al., 2000), and humans (Macfarlan et al., 2014). Studies on these species revealed multiple proximate mechanisms of how access to females can be regulated, who cooperates, and the ontogeny of cooperative relationships. In the following section, I discuss whether these mechanisms might also apply to cooperation in male bottlenose dolphins.

Access to females in non-kin alliances

Reproductive skew

In lions, paternity success was found to be highly skewed towards the dominant male when alliances were small and consisted of relatives (Packer et al., 1991). Paternities in larger alliances consisting of unrelated males in contrast, were less skewed. Lions therefore seem to adjust the degree of access to females depending on the composition of relatives to non-relatives and thus, to the proportion of direct to indirect fitness benefits.

Accounting for direct and indirect fitness benefits, multiple models have been developed to explain differential reproductive success among group members (reviewed in Johnstone, 2000). Transactional models are concerned with group stability and its consequences for division of reproduction (Reeve et al., 1998). Compromise models, such as tug-of war models, in contrast, ignore group stability but view reproductive shares as an outcome of a competition among group members (Cant, 1998; Reeve et al., 1998). In Shark Bay, the number of consortships varied greatly among second-order alliance members (supporting information, Table S4.1). However, more data on second-order alliances to investigate their stability and particularly more comprehensive paternity data are needed to answer which skew model can best explain differential reproductive success in male dolphin alliances. As a first step, however, reproductive skew of multiple alliances could also be inferred using consortship data.

Dominance

Dominance status is often predictive of access to females (Ellis, 1995). In lekking birds, for example, alpha males gain almost exclusive access to females (du Val, 2007). Although an alpha male's unrelated subordinates gain neither direct nor indirect fitness benefits from helping, they benefit from remaining in the lek because they can rise in rank over time (du Val, 2007; McDonald and Potts, 1994).

This queuing system therefore regulates who and at what time males gain access to females, permitting stable cooperation among non-kin. A queuing system contains males of various age classes but I found that male alliances mostly consisted of similarly-aged males (Chapters 2 and 3). In addition, older males did not sire more offspring compared to younger males (Chapter 4) as is expected in a queuing system. Thus, a queuing system is highly unlikely to explain the stability of male alliances in Shark Bay dolphins.

In chimpanzees, top-ranking males, which were not necessarily related, tolerated each other's mating activities but repeatedly cooperated in pairs or trios to prevent other males from mating with females (Watts, 1998). If second-order alliances in dolphins were organised hierarchically, the same mechanism could explain the correlation between first-order alliance stability and consortship success I described in Chapter 4. In such a scenario, the highest-ranking males would repeatedly form first-order alliances within which they shared access to females but obstructed other second-order alliance members from mating. Lower-ranking males might still benefit from remaining in an alliance depending on the availability of females and how well they can be monopolised, the help received from their second-order alliance members, or being allowed to mate with a female when providing help. Furthermore, since second-order alliances mainly consist of unrelated males (Chapters 2 and 3) any existing hierarchy is expected to be flat and reproductive skew to be low so that also low-ranking males would have reproductive opportunities.

To date, it is unknown whether male alliances in dolphins are hierarchical and if dominance could determine access to females. However, a study carried out in captivity found suggestive evidence for dominant-subordinate relationships (Samuels and Gifford, 1997). Whether this is also the case in the wild could be addressed by collecting data on directional changes and whether they tend to be initiated by the same males which can be investigated by UAVs (Lewis et al., 2013b). However, a finding that the same males consistently lead or follow can also be explained by other mechanisms than dominance, for example indirect fitness benefits as found in common bottlenose dolphins (*Tursiops truncatus*) in Florida (Lewis et al., 2013b). As male alliances in Shark Bay mostly consist of unrelated males (Chapter 3), I consider it unlikely that indirect fitness benefits influence directional changes.

Determinants of cooperative partner choice outside of relatedness

Cultural and personality homophily

Homophily of various traits, for example in age and personality, has been shown to influence partner choice in multiple species (Ebenau et al., 2019; Massen and Koski, 2014; McPherson et al., 2001; Mourier et al., 2012). In Chapters 2 and 3 I found that second-order alliances mainly consisted of

similarly-aged males. Apart from homophily in age, homophily in other traits is likely to influence alliance formation among male dolphins. Sponging is a foraging technique in which dolphins use marine sponges as tools for ferreting prey hiding in the seabed. This behaviour is culturally transmitted from mothers to their offspring (Krützen et al., 2005; Wild et al., 2019a). Since sponging is geographically restricted to deeper waters, only a subset of the population are proficient spongers. It has been argued that sponging precludes male alliance formation as it is a time-consuming and solitary technique (Krützen et al., 2005). However, this is not the case. Instead, sponging males are more likely to form alliances with other male 'spongers' (Bizzozzero et al., 2019). Since spongers spend more time foraging, non-sponging adolescent males might have more compatible activity budgets and inevitably, forge bonds to each other. An alternative explanation is that sponging males forge social bonds to each other already as calves because their sponging mothers tend to associate with each other (Kopps et al., 2014b). Based on the results presented in Chapters 2 and 3, both scenarios are possible. Unfortunately, I could not test whether males already forge social bonds to future allies before they were eight years old and thus, the influence of the social network of mothers on the alliance network of their sons is unknown but is currently investigated by another PhD project in our research group. In case the mother's social network influences the choice of allies, sponging males might form alliances not only due to their compatibility but also based on their familiarity as calves. Another research project looks at the influence of personality traits, for example boldness, on the choice of allies in dolphins using boat-based playback experiments that mimick the approach of another alliance or by playing a novel sound.

Partner competence in complex tasks

Cooperative behaviours where individuals selectively interact with certain others in complex tasks were found to be more prevalent in species where average relatedness among group members is low (Lukas and Clutton-Brock, 2018). This finding might not purely be based by low kin availability, since individuals might choose the most competent partner, rather than the closest relative in complex tasks, thereby maximising direct fitness benefits (Chapais, 2006). Complex tasks for example may involve coalitions to either maintain or rise in rank, or gaining access to resources. This contrasts with non-complex tasks, such as huddling for warmth, which are expected to be kin-biased. In chimpanzees, for example, unrelated males participate in border patrols, share meat after hunts, and form coalitions with their close social partners to maintain or rise in rank and thus, increase their reproductive success (Boesch et al., 2006; Mitani et al., 2000; Wroblewski et al., 2009).

Ontogenetic aspects of alliance formation among non-kin

Persisting social bonds from adolescence to adulthood

Male-male relationships in chimpanzees bear some striking similarities when compared to dolphins. Weaving together what we have learned in both species might therefore serve our quest towards understanding the underlying mechanisms of male cooperation and alliance formation among non-kin. Similar to dolphins, bisexual philopatry in chimpanzees permits males to remain in their natal area and form enduring social bonds (Mitani, 2009). Although males of both species would have the possibility to bias social bonds and cooperative behaviours towards kin, this is not always the case. Rather than cooperating with kin chimpanzee males cooperate with their closest social partners (Langergraber et al., 2007; Mitani, 2009; Samuni et al., 2018). In addition, males form coalitions to rise in rank and thereby increase their reproductive success (Boesch et al., 2006; Wroblewski et al., 2009).

Another similarity is the formation of social bonds during adolescence before they play a crucial role in adulthood. A recent study found that in chimpanzees, adolescent and adult males have a comparable number of male-male social bonds. In addition, the social bonds of adolescent males did not differ from the ones found in adult males with regards to social bond strength, as measured by combining patterns of association, proximity and grooming (Sandel et al., 2020). During adolescence, chimpanzees do not yet engage in coalitions or form dominance hierarchies (Sandel et al., 2017). Consequently, the social bonds of adolescent chimpanzees must serve another purpose than reproduction, such as an investment into future coalition partners. The persistence of individual bonds from adolescence into adulthood was not explicitly tested in the chimpanzee study. However, the results imply that as in dolphins, social bonds are formed well before reproduction in order to increase reproductive success. Based on this, it is conceivable to assume that the foundations of unrelated males cooperating are laid early in life. This of course, poses the question of how such a system can evolve in the first place since evolution acts on the phenotype and not on future reproductive success.

The alliance hypothesis in humans

In humans, where non-kin cooperation builds the backbone of societies, the 'alliance hypothesis' proposes that individuals form a network of allies before the onset of conflicts (DeScioli and Kurzban, 2009). Applied to non-human males, forging a network of reliable allies before adulthood would greatly improve a male's ability in intrasexual conflicts once adult. As a consequence, young males with such a network in place may have a competitive advantage which in turn, increases their access to females. Indeed, my results from Chapter 2 and 3 strongly imply that social bonds to future alliance members are already forged during adolescence. Benefits of maintaining such bonds potentially include high levels of familiarity and a better ability to coordinate behaviours. However, such a

strategy can also be risky. If an adolescent male loses his close associates, or part thereof, he might be less embedded within the social network of a second-order alliance once adult and therefore, engage in fewer consortships. Although this is unlikely since mortality rates are highest in dependent calves (King et al., 2015), we know of one case where a male lost his close associates during adolescence (unpublished data). Said male has not yet established second-order alliance membership despite being in his late teens by now.

Alliances as persisting all-male groups or bachelor herds

All-male groups are commonly observed in species where females form their own groups or harem-forming species where groups consist of more females than males. Such all-male groups can either consist of males of multiple age classes (e.g., elephants, *Loxodonta africana*, Chiyo et al., 2011) or of young, not yet sexually active males, also referred to as 'bachelor herds' (e.g., zebra, *Equus zebra*, Penzhorn, 1979). Within all-male groups, young males benefit from associating with their same-sex peers as it allows them to prepare for male-male competition with a lower risk of getting injured. Male elephants, for example, sparred preferably with those of similar age (Chiyo et al., 2011). Likewise, play-herding, a behaviour where male dolphins practice consortship behaviour, is observed among adolescents (Connor et al., 2000b).

In contrast to calves, male juvenile dolphins associate more with males than females (Krzyszczyk et al., 2017), suggesting at least some level of segregation between the sexes upon reaching independence. Alliances among similarly-aged males could have evolved by males primarily associating with their peers in all-male groups and maintaining these associations throughout their lives, rather than joining a breeding group. While initially, associating with other males might have protected young males from threats and allowed them to hone skills needed in adult life, the maintenance of social bonds to other males could have increased their competitive potential as adults and thus, increased their access to females. Due to the reproductive advantage of males maintaining their social bonds to others, this behaviour might have been positively selected for. In bottlenose dolphins, all-male groups could thereafter have served as stepping stones towards the evolution of male alliances.

The alliance gradient in bottlenose dolphins and its explanations

Various degrees of male alliance complexity

In contrast to male chimpanzees, where coalition formation is observed across different communities and habitats (Gilby et al., 2013; Nishida and Hosaka, 1996; Watts, 1998), alliance formation does not seem a universal trait in bottlenose dolphins (*Tursiops* spp.). In Sarasota Bay, Florida, similarly-aged common bottlenose dolphin (*T. truncatus*) males form dyadic first- but not second-order alliances (Wells, 2014). This contrasts the St Johns River common bottlenose dolphin population, located on the opposite side of the Florida peninsula, where males potentially form nested second-order alliances (Ermak et al., 2017), although at a lower percentage compared to Indo-Pacific bottlenose dolphins (*T. aduncus*) in Shark Bay (Widrick, 2016). However, in contrast to Shark Bay, second-order alliances in the St Johns River population were based on association indices without including any functional criteria (Connor and Krützen, 2015). In two common bottlenose dolphin populations in temperate waters, Moray Firth, Scotland, and Doubtful Sound, New Zealand, dolphin males (*T. truncatus*) preferably associate with certain others, but alliances have not been detected despite long-term behavioural data collection (Eisfeld and Robinson, 2004; Lusseau et al., 2003; Wilson, 1995).

Various degrees of alliance formation can also be observed in Indo-Pacific bottlenose dolphin populations (*T. aduncus*) along the Western Australian coast. Shark Bay marks currently the northernmost population where long-term behavioural data has been collected. Approximately 800 kilometres south, in the Swan Canning Riverpark close to Perth, males form first-order alliances with similarly-aged others but second-order alliances are not evident (pers. comm. Delphine Chabanne). Further south along the coast in Bunbury, it is unclear whether males actually form alliances or not (Sprogis et al., 2016).

Various degrees of relatedness in male alliances

Apart from the propensity to form alliances at various degrees, males also differ in their means to form alliances with kin. In the Bahamas (*T. truncatus*, Parsons et al., 2003) and South Australia (*T. cf. australis*, Diaz-Aguirre et al., 2018), alliances were more closely related than expected by chance, although both had some methodological issues. In South Australia, additional studies are needed to confirm that male groups actually serve to increase access to females while in the Bahamas, the selected reference population to calculate relatedness estimates was potentially unsuitable. Kinship did not explain alliance formation in Sarasota (*T. truncatus*, Wells, 2014), south-eastern Australia (*T. aduncus*, Möller et al., 2001), and Shark Bay (*T. aduncus,* Chapters 2-4). Thus, the formation of kin-biased alliances is neither confined to a species nor a specific region.

Dolphins occupy a large range of marine habitats (Leatherwood and Reeves, 2012). The formation of male alliances in general, but also the formation of kin-biased alliances, might thus reflect adaptations to their respective social and ecological environments. If we aim to understand what drives the formation and evolution of male alliances in dolphins and why kin selection can explain alliance formation in some populations but not in others, it is important to identify traits which appear to promote or hinder alliances in general, as well as the ones impeding or stimulating kin-biased alliances. Dolphin populations vary in patterns of dispersal ability, site fidelity, life history parameters, and population size and density. Each of these variables is likely to influence whether and among whom alliances are formed. Here, I will discuss the potential influence of these variables on male alliance formation, attempting to construe the unique combination of variables allowing the emergence of multi-level alliances among unrelated males in Shark Bay.

Variables influencing alliance formation and kin-biased partner choice

Philopatry

Dispersal, *i.e.*, leaving the natal area for the purpose of breeding, is usually constrained to members of one sex, in mammals predominantly males (Greenwood, 1980). Sex-biased dispersal limits the opportunity to form persisting social bonds in the dispersing sex. As a consequence, social bonds are more prevalent in females, compared to male mammals (Silk, 2007a). Apart from dispersal, social bonds are also harder to maintain where individuals show seasonal migration patterns. In Sarasota as well as in Bunbury, the ranging patterns of dolphins change throughout the year (Fazioli et al., 2006; Sprogis et al., 2016) and in south-eastern Australia, males disperse (Möller and Beheregaray, 2004; Wiszniewski et al., 2010). It would be interesting to investigate whether alliance partnerships in these populations are also foreshadowed by social relationships during adolescence and if closely bonded

males disperse together as it is the case in certain primate species (Schoof et al., 2009). Based on my finding that associations in early life foreshadow alliance partners in adulthood (Chapters 2 and 3), it is likely that close associates jointly disperse and become alliance partners.

In general, members of the philopatric sex are expected to be more closely related than of the dispersing sex (Prugnolle and de Meeus, 2002). Philopatry therefore facilitates the formation of social bonds with kin. For example in cercopithecine primates, where females are philopatric, females forge social bonds to their female relatives and cooperate within matrilines (Silk, 2009). In Shark Bay, dolphins are observed at similar rates year-round and are bisexually philopatric (Krützen et al., 2004b). However, while relationships on kinship have been documented in females (Frère et al., 2010b), neither cooperative partner choice nor the persistence of social bonds in males was explained by relatedness patterns (Chapters 2 and 3).

Generally, females benefit from associating with others through facilitated detection of food and predators, lower risks of male harassment, as well as assistance in the rearing of their offspring (Clutton-Brock, 2016). These tasks can be fulfilled by most others and are easiest to coordinate among individuals with shared energetic needs. Indeed, female dolphins primarily associate with their dependent calves and females in the same reproductive state (Möller and Harcourt, 2008; Smolker et al., 1992). Possibly as a consequence of their changing reproductive states, the social bonds of females are less stable compared to the persisting social bonds of males. The more fluid relationships of females allows them to associate with kin of all age classes, while this may not be the case for males.

Alliance size

In mammals, maternal half-siblings are often preferred partners (Smith, 2014) and can be reliably recognised through familiarity when phenotypic mechanisms to discriminate kin from non-kin are absent. The long interbirth intervals of four years in Shark Bay (Mann et al., 2000a) suggest that very few males have similarly-aged half-brothers. The likelihood to have a maternal half-brother is further diminished by the high calf mortality of 44% (Mann et al., 2000a). Given the low kin availability, the likelihood for forming kin-biased alliances further decreases with alliance size. Even if dolphins were able to distinguish paternal relatives, the highly promiscuous mating system (Connor and Krützen, 2015) combined with low paternity skew (Krützen et al., 2004a) is likely to result in male dolphins having incongruent sets of relatives, posing a 'stable roommate problem'. Stable roommate problems deal with how groups are optimally created while adhering to each individual's preferences (Gale and Shapley, 1962). Under kin selection, partner preference depends on relatedness. In the case of dolphins, assume a scenario where A and B are half-siblings. However, A's second half-brother C, is unlikely to also be B's half-brother. In turn, B's half-brother D, is probably unrelated to A or C, creating

a matching problem of which trio should form an alliance The formation of polyadic alliances based on relatedness would be equally problematic and hence, alliance formation might underlie other traits. Interestingly, humans and chimpanzees, where individuals also do not have matching sets of relatives between individuals, were found to prefer relatives in dyadic, but not in polyadic settings (Nolin, 2011; Suchak et al., 2014).

Skewed operational sex ratio

The highly skewed operational sex ratio in Shark Bay dolphins is owed to the combination of maternal care, a late age of first reproduction and large interbirth intervals (Mann et al., 2000a). Since multiple males are expected to outcompete a single one, the costs of shared matings can be outweighed by the benefit of cooperative defence of females (Connor and Whitehead, 2005). Taking into account life history data, such as calf mortality rates and interbirth intervals, is therefore crucial to increase our understanding of female availability and thus, the extent male-male competition.

Population density

In dense populations where encounter rates are high, males are unable to consort a female over a longer period of time without being contested by other males. Assuming that males increase their competitive potential when cooperating with others, multiple males might outcompete a single one and larger alliances are likely to outcompete smaller ones (Whitehead and Connor, 2005).

Population density and encounter rates are also likely to affect the complexity of alliances. Complex social structures can emerge where individuals recognise each other and selectively and repeatedly cooperate with certain others out of a pool of potential partners (Kappeler, 2019). This complexity can be further enhanced when interactions exceed the dyad because it creates opportunities for audience effects (Zuberbühler, 2008), eavesdropping (Valone, 2007), coalitionary intervention (Harcourt and de Waal, 1992), conflict management by policing (Flack et al., 2005), and other third party interactions. Considering that dolphins have high cognitive capabilities (Fox et al., 2017; Marino et al., 2007) it is likely that they recognise not only each other but also third-order relationships and therefore, are capable to execute such complex behaviours given the right circumstances. Compared to other populations Shark Bay has a high population density (Connor et al., 2000b; Nicholson et al., 2012). This high population density including high encounter rates might have permitted the evolution of complex, multi-level alliances consisting of up to 14 males.

Food availability

High population densities can only be maintained where adequate food supply is available. Generally, in habitats of higher quality individuals are expected to spend less time foraging and feeding (Li and

Rogers, 2004; Tremblay et al., 2005). In addition, the costs of aggregation are decreased due to lower levels of competition. Consequently, individuals form larger groups and spend more time socialising in habitats where food availability is high compared to such of low productivity (Roth et al., 2020; van Schaik, 1999). Multi-level societies are usually observed in species where population size is not limited by food resources (Grueter et al., 2017). To unravel the foundations of male alliance formation in bottlenose dolphins, it is therefore crucial to compare various habitats in terms of their food availability.

Predicting male alliance formation in multiple habitats

To assess the influence of all the aforementioned variables on male alliance formation, they would need to be compared to other populations with a similar genetic background as to avoid genetic confounds, but across multiple habitats. This is currently done by Manuela Bizzozzero who compares the Eastern and Western gulfs of Shark Bay. Thus, potential differences in alliance formation such as second-order alliance size, alliance stability and other social network metrics, or the overall number of second-order alliances between the two subpopulations are likely to reflect variation in habitat and/or distribution of females. Over the last four decades, each study site extension revealed either a new alliance level or behaviour, the propensity to consort in pairs or trios, for example (Connor et al., 2017; Connor and Krützen, 2015). Thus, characteristics of alliances can, indeed, vary over such a small geographical scale. Habitat differences might, among others, include abundance of prey and predator species and also influence the distribution of receptive females. Disentangling the interplay of these variables can therefore shed light on determinants of male alliance formation. In a second step, different patterns of male alliance formation could be investigated in terms of ecological and population specific parameters along the Western Australian coastline where populations have shared a very recent evolutionary history (S. Witter, pers. comm) but appear to differ in levels and complexity of alliance formation.

On a more temporal scale, the ecological effects on male alliance formation can also be studied where long-term field sites experienced a change in habitat. In Shark Bay for example, an unprecedented heatwave in 2011 led to a catastrophic decline of seagrass meadows (Arias-Ortiz et al., 2018). In the Western gulf, we found survival rates of dolphins and the number of calves per year to be lower after the heatwave compared to the years before (Wild et al., 2019b). How this will affect alliance formation will only be revealed in a few years. Two scenarios are possible: either alliances become smaller because fewer similarly-aged males are available to choose as allies or alternatively, alliances will be similar in size but no longer consist of similarly-aged males.

Potential methodological issues

No nepotism in male alliances or incorrect relatedness estimates?

To investigate the effect of relatedness on male alliance formation, I estimated pairwise relatedness from 9,991 informative SNPs. This genomic approach is expected to result in more accurate relatedness estimates compared to the up to 27 microsatellite markers used in earlier studies. To verify this assumption, Samuel Wittwer and I simulated dyads of unrelated individuals, half-siblings, and parent-offspring in Coancestry (Wang, 2011). We did this 1,000 times for each relationship category for both, the 27 microsatellite markers and the 9,991 SNPs. The SNPs proved to be closer to the expected value for these relationships and also showed a narrower distribution around the expected value, overall suggesting that our SNPs outperformed the microsatellite markers (Fig. 5.2).

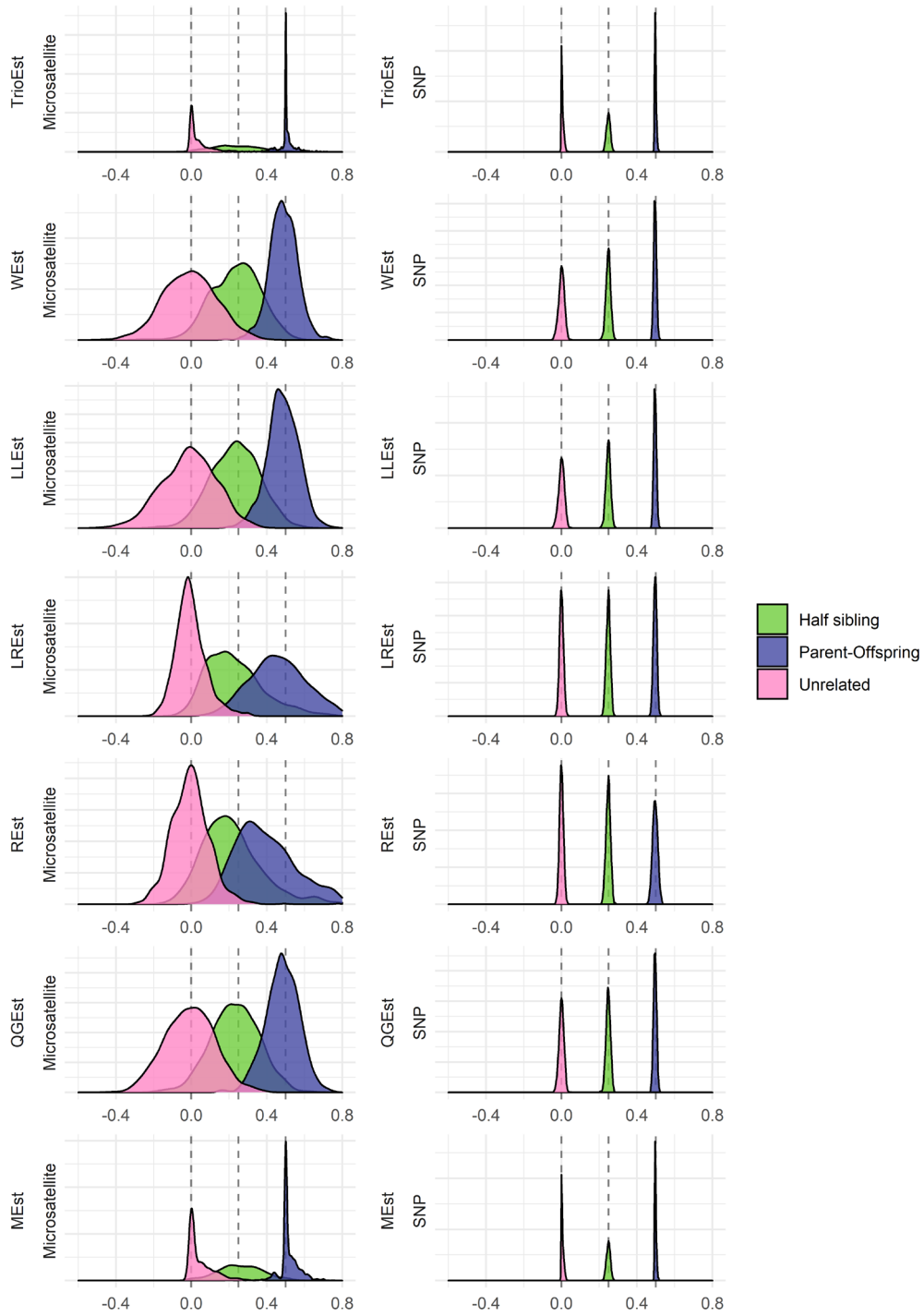


Figure 5.2. Distribution of simulated dyads of the following relationships: unrelated, half-siblings, parent-offspring with the dashed line denoting the expected relatedness value per category (0, 0.25, 0.5). We simulated dyads for all seven relatedness estimators included in Coancestry (Wang, 2011). The analyses in the data chapters were carried out using the TrioEst multilikelihood estimator (Wang, 2007). The wider distribution observed in the simulations based on 27 microsatellites (left half) compared to 9,991 SNPs (right half) suggests that the ddRAD approach produces more accurate relatedness estimates over all estimators.

Although male dolphins in Shark Bay are philopatric, I only found a small number of available kin to choose as allies (Chapter 3). Furthermore, levels of relatedness were low across all males (min = 0, max = 0.577, mean = 0.014 ± 0.040, Figure 5.3).

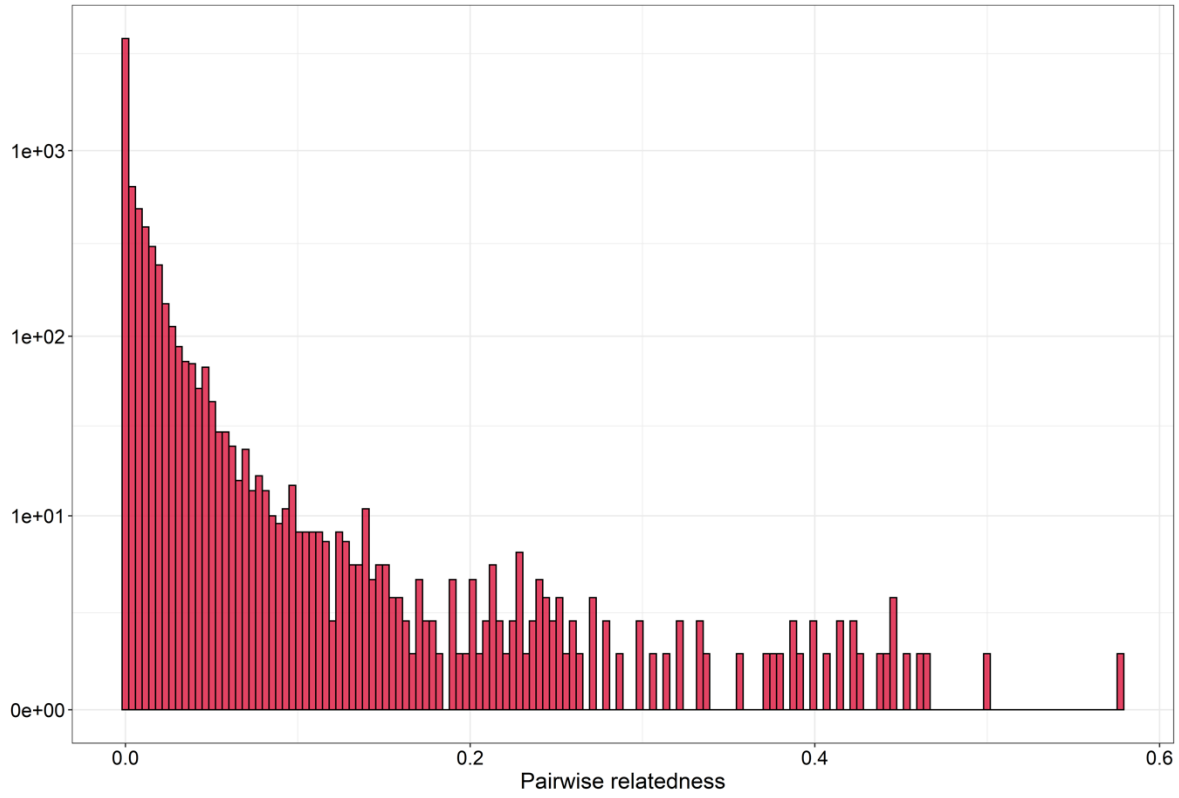


Figure 5.3. Pairwise relatedness values between all males (N = 120) for which relatedness estimates based on ddRAD data was available. Most dyads were unrelated and only a few dyads are related on the level of half-siblings or more (r > 0.25). Note the logarithmic scale.

This low level of relatedness in the philopatric sex imposes the question whether our relatedness estimates are accurate or not. A study demonstrated that a large number of same-sex relatives is only available in small groups where reproduction is limited to a few individuals (Lukas et al., 2005). Preliminary analyses suggest that paternity concentration among Shark Bay males is low (Krützen et al., 2004a). Thus, my finding of highly limited kin availability in the relatively large and open fission-fusion society in Shark Bay is not surprising.

Accuracy of relatedness estimates from ddRAD data can, amongst other factors, be influenced by genotyping error, allelic dropouts and the number of SNPs (Attard et al., 2018; Foroughirad et al., 2019). We obtained close to 10,000 SNPs, more than double the amount of what was found to restrict the false classification of dyads to various relationship categories to less than 5% (Foroughirad et al., 2019). The effect of genotyping error and allelic dropouts mostly leads to a slight downward bias of

relatedness estimates (Attard et al., 2018). As most analyses in my work involving genomic data were of correlative nature, the general patterns of my results would remain, even if the genomic data were slightly biased.

In the next years, we are likely to see improvements on relatedness estimations in non-model species from genomic data based on longer scaffold reads, whole genome data and annotated reference genomes. All of these will provide researchers working on non-model organisms with a with a better idea on potential linkage between loci and permit to differentiate between sequences that are identical by descent from such that are identical by state. Both will further allow us to classify dyads to specific relationships categories and to reconstruct multi-generational pedigrees (Albrechtsen et al., 2009; Staples et al., 2014).

Assessing social bonds in bottlenose dolphins

In my thesis, I estimated social bond strength via patterns of association which are quantitative and thus, might not capture qualitative aspects of relationships. Considering the abundance of reasons why two individuals affiliate, the inference of social bonds via patterns of associations appears to be a crude measurement. It has been proposed that estimates of social bond strength should not be solely based on association patterns but be measured through a combination of variables which in primates, often include associations, proximity, and grooming (Silk et al., 2013). Having access to multiple measures, including one on relationship quality, should also be the preferred option in bottlenose dolphins.

Two behaviours that might reflect relationship quality are petting and synchronous surfacing. Synchronous behaviours evoke the release of endorphins (Tarr et al., 2016) and oxytocin (Jiang and Platt, 2018; Spengler et al., 2017), which promotes bonding and facilitates cooperative relationships (chimpanzees, Crockford et al., 2013; humans, de Dreu et al., 2010; dogs, *Canis familiaris*, Romero et al., 2014). Since oxytocin is universal to mammals, it could well be that these effects are shared in dolphins. However, a recent study on the Shark Bay dolphin population found that in some alliances, the degree of synchrony appears to be inversely related to association strength (McCue et al., 2020). This was unexpected and interpreted as males attempting to achieve higher degrees synchrony. Nevertheless, future work on the quality of social bonds in bottlenose dolphins should include detailed behavioural data on petting (initiation, duration) and degree of synchrony among males.

Studies in the near future will be able to include additional measures to measure social bond strength than patterns of associations. The usage of UAVs increases our ability to collect data on petting and synchronous behaviours, and methods to assess the concentration of stress hormones in small

cetaceans are being established (Kellar et al., 2015). While these additional measures will allow us to assess the multiple dimensions of social bonds, such as predictability, symmetry, and stability (Silk et al., 2013), I expect that they will not contradict our measures of social bond strength based on association patterns alone. Allies are characterised by high levels of association which are well above random. Furthermore, allies associate in various contexts including foraging, travelling, resting, and socialising (Connor et al., 1992). Thus, association patterns are not confounded to reflect social partners in a single context only. In all chapters, I only included well-known males that were seen often, reducing the likelihood to wrongly attribute a dyad as closely bonded or to miss a male's social partner.

Outlook

Shared among all three data chapters is the high relevance of social bonds which are formed independent of relatedness. Social bonds predicted partner choice on both alliance levels and affected a male's number of consortships and thereby probably his reproductive success. The next steps will be to unravel more details on the mechanisms involved in the formation and maintenance of cooperative partnerships.

Ontogeny and heritability of social network position

From Chapter 4, we have learned that social position within the second-order alliance is likely to influence reproductive success. While we know from Chapters 2 and 3 that dyadic social bonds during adolescence foreshadow alliance partners, we not yet know if adolescent social network position predicts social network position in adulthood and thus, whether well-connected adolescents become well-connected adults. In long-tailed manakins (*Chiroxiphia linearis*) for example, juvenile network position predicted future network position (McDonald, 2007). Whether this is also the case in bottlenose dolphins is currently studied by Kathryn Holmes.

Future studies should also aim to explore the effect of social network position in different contexts. In humans, for example, well-connected individuals achieve higher reproductive success but at the same time, are more prone to sickness (Page et al., 2017). In bottlenose dolphins, individuals with higher centrality might obtain more offspring but have a shorter life span as they are more likely to catch a disease such as the fatal cetacean morbilivirus (Stone et al., 2011).

In species where individual differences in sociality affect reproductive success, the propensity to forge social bonds to others might be under genetic control. That social network attributes are heritable was found in humans and rhesus macaques (*Macaca mulatta*) (Brent et al., 2013; Fowler et al., 2009). Whether this is also the case in other species, particularly outside of the primate lineage, has to the best of my knowledge, not yet been explored.

Differential reproductive success between second-order alliances

In Chapter 4 I used consortship data spanning nearly two decades and could show that consortship success varies among second-order alliance members. I found that within second-order alliances, consortship success is influenced by social bonds to second-order alliances members as well as first-order alliance stability (Chapter 4). Future studies on potential dominance-subordinate relationships, paternity skew, juvenile social network position, and body size, will shed light on the underlying traits and mechanisms influencing differential fitness within second-order alliance members. However,

future studies should also aim to elucidate why consortship success differs between second-order alliances (Connor et al., 2017; Hamilton et al., 2019).

In contrast to other species where individuals of both sexes are organised in clearly recognisable linear dominance hierarchies and individuals cooperate to either maintain or rise in social rank, intra-alliance relationships in bottlenose dolphins do not seem to follow this pattern and severe fights among allies are rarely, if ever, observed. However, conflicts are observed between second-order alliances (Connor and Krützen, 2015). Rather than individual ranks within alliances, second-order alliances could be the basic hierarchical unit in bottlenose dolphins. If that is the case, some second-order alliances are expected to consistently outcompete others in terms of number of consorted females and/or direct reproductive output.

The idea that groups can be hierarchical units is the basis of the intergroup dominance hypothesis (Crofoot and Wrangham, 2010) and social dominance theory (Sidanius and Pratto, 2001) but to date, both have rarely been tested and studies appear to be confined to primates and humans. While this might pertain that the importance of between-group conflicts on fitness is low (Cowlishaw, 1995) the reason of lack of evidence is likely to also be of empirical nature. Behavioural data collection on between-group interactions are difficult to obtain since many individuals interact simultaneously. Hence, data on multiple individuals needs to be collected simultaneously and reliably to record who initiated attacks, who responded and how. Such detailed behavioural data is close to impossible to collect using traditional behavioural data collection such as focal follows or group scans. However, recent technological advances permit the usage of UAVs to collect and quantify detailed data on collective animal behaviours. This includes the simultaneous recording of behavioural states and body orientation of all group members multiple times per second, even including their head positions (Graving et al., 2019).

The fact that UAVs are highly promising tools to study animal behaviour has also been recognised by cetacean researchers which moved skywards in the past years instead of diving into the water (Fiori et al., 2017; Fiori et al., 2020). Other than UAVs, telemetry tags on a single individual per group allows the study of multiple groups relative to each other (Crofoot et al., 2008), thereby providing insights into which groups avoid or approach each other. Combining data on group movement patterns with information on the distribution of critical resources such as mates, food, and predator activity fields, yields additional insights on which groups reign over valuable habitats and which groups are limited to less favourable habitats (Willems et al., 2009; Willems and Hill, 2009). Since inter-alliance conflicts cannot be observed on a frequent basis, data on inter-alliance conflicts could also be artificially generated by imitating the approach of a second-order alliance when observing another one via play-

back experiments and measuring the behavioural response, *i.e.*, leaving or approaching. The combination of any of the approaches to assess inter-alliance conflicts with paternity data will enable us to assess the influence of second-order alliance membership on fitness.

Social bonds and their influence on stress and longevity

In terms of adult male careers, I could demonstrate which aspects of social bonds, namely first-order alliance stability and social bond strength to second-order alliance members, influenced consortship success. However, social bonds have been demonstrated to not only increase access to mates but to increase life span (Archie et al., 2014; Barocas et al., 2011; Holt-Lunstad et al., 2010; Silk et al., 2010b). The positive effect of social bonds on lifespan were attributed by social bonds decreasing stress (Young et al., 2014a) or increasing immune responses and health (Capitanio et al., 1998). Considering the slow life history of male dolphins including a long reproductive period, longer lives are expected to positively affect fitness by prolonging an individual's reproductive period.

Future research projects exploring various aspects of social bonds and their effect on life span in male dolphins will provide valuable insights into how social bonds shape male careers throughout life in male dolphins, including their effect on longevity. In the best case, this behavioural data will be combined with physiological data measuring stress to investigate how social bonds affect stress and health of male dolphins.

Sample collection to determine hormonal profiles proves to be incredibly difficult in small marine mammals: urine cannot be collected and compared to whale blow, the blow of dolphins is much smaller and close to impossible to obtain in the wild. Blubber samples in contrast, are routinely collected for DNA analysis and allow us to assess stress by measuring the concentrations of stress hormones or via DNA methylation patterns. Blubber cortisol levels have recently been demonstrated to predict chronic physiological stress in humpback whales (Mingramm et al., 2020). In dolphins, cortisol levels were successfully determined from blubber and revealed to be related to recent stress (Kellar et al., 2015).

Over the last years, DNA methylation patterns were found to hold information on stress and resilience (Klengel et al., 2014; Zannas and West, 2014). DNA methylation patterns thus, appear to be a promising tool to assess stress from blubber or tissues samples. While this is still a new field of research and most studies were conducted in controlled laboratory conditions (Elliott et al., 2010; Provençal et al., 2012), studies have also been carried out in the wild. In free-living tree swallows (*Tachycineta bicolor*), differences in methylation patterns were related to stress resilience (Taff et al., 2019). In baboons, it was demonstrated that individuals entirely feeding in the savanna had a different

methylation pattern compared to such having access to human food scraps. Interestingly, these methylation patterns persisted even when individuals dispersed, suggesting a lasting change in gene expression depending on early life environment (Lea et al., 2016). DNA methylation patterns can therefore provide us with valuable insights on the interplay between ecological variables and the genome.

In Shark Bay, the combination of life history data with social networks and epigenetic data on DNA methylation, will allow us to assess whether differences in social network position are reflected in methylation patterns. This places us in the position to investigate whether the number of social bonds is related to stress or a shorter life span, reducing lifetime fitness.

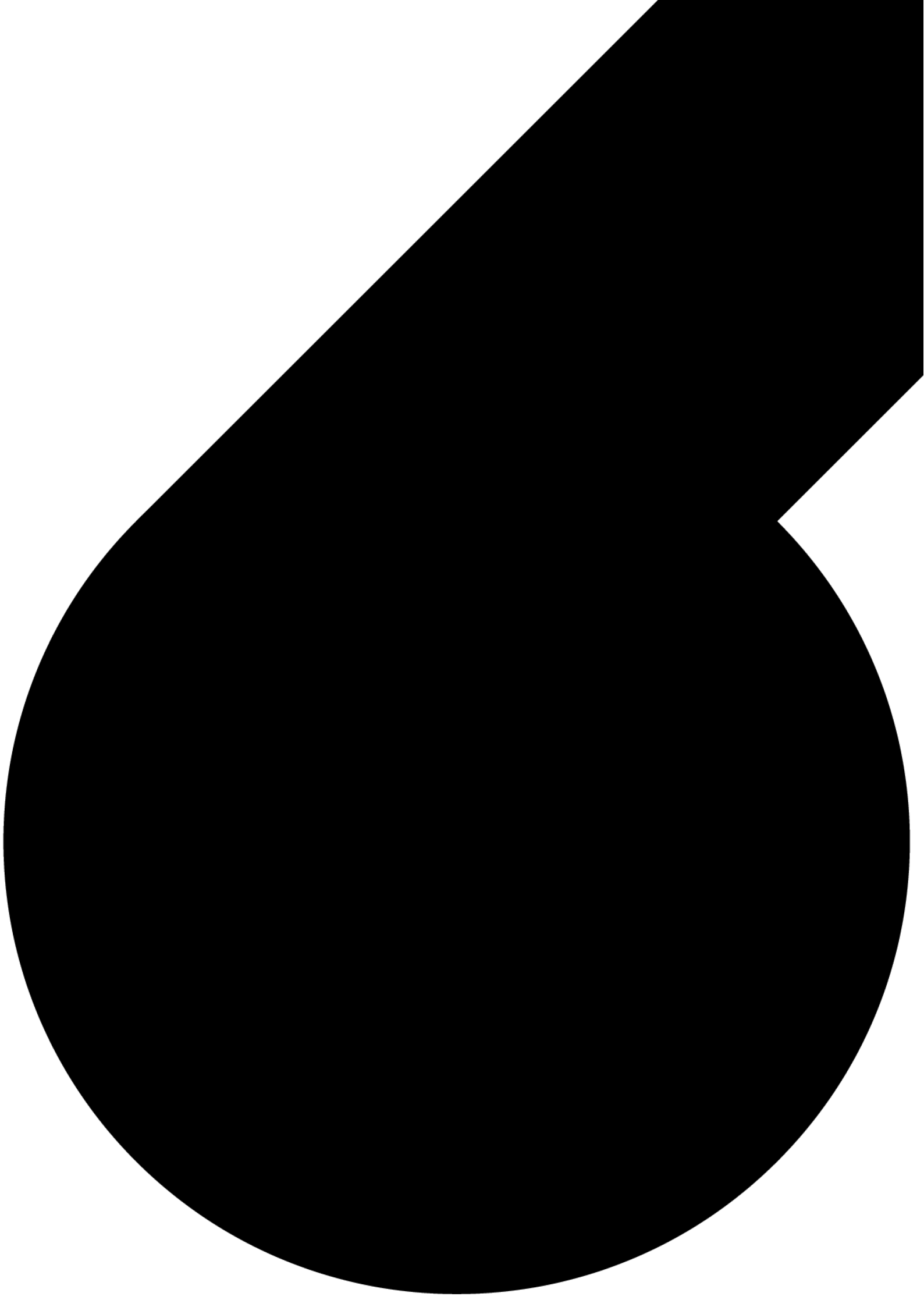
An almost incredible coincidence is the finding that accurate age estimates can be gained from DNA methylation patterns (de Paoli-Iseppi et al., 2017; Polanowski et al., 2014). While this has not yet been tested in the Shark Bay dolphin population, a verification of this method is underway and was successfully tested in another dolphin population (Beal et al., 2019). Since we lack accurate age estimates on multiple males of which we have ample behavioural data on, methylation data could vastly increase our sample size. Furthermore, the usage of methylation patterns in populations where alliances are observed but age data is largely missing such as in the Western gulf of Shark Bay, permits us to investigate if alliances consist of similarly-aged males as observed in the Eastern gulf (Chapter 3).

I am confident that continued behavioural and genetic data collection on multiple dolphin populations will provide us with additional pieces to the puzzle of male cooperation on both, the ultimate and proximate level. The different degrees of alliance formation observed across the globe and identifying variables predicting the formation of multi-level societies will increase our knowledge on the evolution of various social systems. In general, a deeper understanding of male cooperation in dolphins is of great interest as it provides valuable insights into the evolution of complex societies comparable to our own.

Conclusion

Taken together, my findings from Chapters 2-4 suggest that social bonds in male dolphins serve a political function: due to the persistence of social bonds (Chapter 2), investment into social bonds in adolescence results in second-order alliance formation adulthood (Chapter 3). Social bonds facilitate alliance formation on both alliance levels (Chapter 3), with well-connected adult males enjoying higher reproductive success (Chapter 4). Social bonds in male dolphins are thus connected to direct and not indirect fitness benefits. This stands in contrast to what is known in many other species where males form alliances.

Through a combination of long-term behavioural and genetic data collected on the renowned Shark Bay dolphin population, the work presented here marks to date, one of the most comprehensive efforts to investigate male alliances. I demonstrate that complex, multi-level alliances where cooperation is not kin-biased can also be found in a marine environment and are not exclusive to the primate lineage or terrestrial animals. Formed in early life among similarly-aged males, male dolphins preferably associated and cooperated with the ones they were most closely bonded to. Using an individual-based approach, I was able to investigate alliance partner choice on both levels and found no effect of relatedness but social bond strength. Furthermore, I could demonstrate that in contrast to multiple asocial variables, a male's social bond strength to his alliance members and partner fidelity to first-order alliance partners predicted reproductive success. Considering that social bonds directly influence fitness, we now understand why males invest into social skills and social bonds to others throughout their lives. My findings on male bottlenose highly resemble the findings of studies carried out in humans. Humans are well-known for their persisting relationships to non-relatives, *i.e.,* 'friendships' and their large-scale cooperation in complex societies. Thus, complex societies based on long-term affiliations seem to have evolved multiple times in different environments, representing a compelling case of convergent evolution.



6. References

Aktipis A, Maley CC, 2017. Cooperation and cheating as innovation: insights from cellular societies. Philos Trans R Soc Lond B Biol Sci 372:20160421.

Alberts SC, 2019. Social influences on survival and reproduction: Insights from a long-term study of wild baboons. Journal of Animal Ecology 88:47-66. doi: 10.1111/1365-2656.12887.

Albrechtsen A, Sand Korneliussen T, Moltke I, van Overseem Hansen T, Nielsen FC, Nielsen R, 2009. Relatedness mapping and tracts of relatedness for genome-wide data in the presence of linkage disequilibrium. Genetic Epidemiology 33:266-274. doi: 10.1002/gepi.20378.

Allen SJ, Bryant KA, Kraus RHS, Loneragan NR, Kopps AM, Brown AM, Gerber L, Krützen M, 2016. Genetic isolation between coastal and fishery-impacted, offshore bottlenose dolphin (*Tursiops* spp.) populations. Molecular ecology 25:2735-2753. doi: 10.1111/mec.13622.

Ames K, Majolo B, Hall K, Wilson N, Brumpton R, Garratt R, 2006. Human friendship favours cooperation in the Iterated Prisoner's Dilemma. 143:1383. doi: https://doi.org/10.1163/156853906778987506.

Amici F, Kulik L, Langos D, Widdig A, 2019. Growing into adulthood—a review on sex differences in the development of sociality across macaques. Behavioral Ecology and Sociobiology 73:18. doi: 10.1007/s00265-018-2623-2.

Amos WB, Twiss S, Pomeroy PP, Anderson SS, 1993. Male mating success and paternity in the grey seal, *Halichoerus grypus*: a study using DNA fingerprinting. Proceedings of the Royal Society B: Biological Sciences 252:199-207.

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA, 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. Nature Reviews Genetics 17:81-92. doi: 10.1038/nrg.2015.28.

Antal T, Ohtsuki H, Wakeley J, Taylor PD, Nowak MA, 2009. Evolution of cooperation by phenotypic similarity. Proceedings of the National Academy of Sciences 106:8597-8600. doi: 10.1073/pnas.0902528106.

Archie EA, Tung J, Clark M, Altmann J, Alberts SC, 2014. Social affiliation matters: both same-sex and opposite-sex relationships predict survival in wild female baboons. Proceedings of the Royal Society B: Biological Sciences 281. doi: 10.1098/rspb.2014.1261.

Arias-Ortiz A, Serrano O, Masqué P, Lavery PS, Mueller U, Kendrick GA, Rozaimi M, Esteban A, Fourqurean JW, Marbà N, Mateo MA, Murray K, Rule MJ, Duarte CM, 2018. A marine heatwave drives massive losses from the world's largest seagrass carbon stocks. Nature Climate Change 8:338-344. doi: 10.1038/s41558-018-0096-y.

Asakawa-Haas K, Schiestl M, Bugnyar T, Massen JJM, 2016. Partner Choice in Raven (*Corvus corax*) Cooperation. PLoS One 11:e0156962. doi: 10.1371/journal.pone.0156962.

Attard CRM, Beheregaray LB, Möller LM, 2018. Genotyping-by-sequencing for estimating relatedness in nonmodel organisms: Avoiding the trap of precise bias. Molecular Ecology Resources 18:381-390. doi: 10.1111/1755-0998.12739.

Auger AP, Olesen KM, 2009. Brain Sex Differences and the Organisation of Juvenile Social Play Behaviour. Journal of Neuroendocrinology 21:519-525. doi: 10.1111/j.1365-2826.2009.01871.x.

Bacher K, Allen S, Lindholm AK, Bejder L, Krützen M, 2010. Genes or Culture: Are Mitochondrial Genes Associated with Tool Use in Bottlenose Dolphins (*Tursiops* sp.)? Behavior Genetics 40:706-714. doi: 10.1007/s10519-010-9375-8.

Barclay P, 2016. Biological markets and the effects of partner choice on cooperation and friendship. Current Opinion in Psychology 7:33-38. doi: https://doi.org/10.1016/j.copsyc.2015.07.012.

Barocas A, Ilany A, Koren L, Kam M, Geffen E, 2011. Variance in centrality within rock hyrax social networks predicts adult longevity. PLoS One 6:e22375-e22375. doi: 10.1371/journal.pone.0022375.

Barrat A, Barthélemy M, Pastor-Satorras R, Vespignani A, 2004. The architecture of complex weighted networks. Proceedings of the National Academy of Sciences 101:3747. doi: 10.1073/pnas.0400087101.
Barton K, Barton MK, 2018. Package 'MuMIn'.
Bates D, Mächler M, Bolker B, Walker S, 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1-48.
Beal A, Kiszka J, Wells R, Eirin-Lopez JM, 2019. The Bottlenose dolphin Epigenetic Aging Tool (BEAT): a molecular age estimation tool for small cetaceans. Frontiers in Marine Science 6:561.
Bercovitch FB, 1989. Body size, sperm competition, and determinants of reproductive success in male savanna baboons. Evolution 43:1507-1521.
Berghänel A, Ostner J, Schröder U, Schülke O, 2011. Social bonds predict future cooperation in male Barbary macaques, Macaca sylvanus. Animal Behaviour 81:1109-1116. doi: 10.1016/j.anbehav.2011.02.009.
Bergmüller R, Johnstone RA, Russell AF, Bshary R, 2007. Integrating cooperative breeding into theoretical concepts of cooperation. Behavioural processes 76:61-72. doi: 10.1016/j.beproc.2007.07.001.
Bigg M, Olesiuk P, Ellis GM, Ford J, Balcomb KC, 1990. Social organization and genealogy of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. Report of the International Whaling Commission 12:383-405.
Bizzozzero MR, Allen SJ, Gerber L, Wild S, King SL, Connor RC, Friedman WR, Wittwer S, Krützen M, 2019. Tool use and social homophily among male bottlenose dolphins. Proceedings of the Royal Society B: Biological Sciences 286:20190898.
Boehm C, 2009. Hierarchy in the forest: The evolution of egalitarian behavior. Cambridge, MA, USA: Harvard University Press.
Boesch C, Kohou G, Néné H, Vigilant L, 2006. Male competition and paternity in wild chimpanzees of the Taï forest. American Journal of Physical Anthropology 130:103-115. doi: 10.1002/ajpa.20341.
Borgeaud C, Bshary R, 2015. Wild Vervet Monkeys Trade Tolerance and Specific Coalitionary Support for Grooming in Experimentally Induced Conflicts. Current Biology 25:3011-3016. doi: https://doi.org/10.1016/j.cub.2015.10.016.
Bourke AFG, 2014. Hamilton's rule and the causes of social evolution. Philos Trans R Soc Lond B Biol Sci 369. doi: 10.1098/rstb.2013.0362.
Braun A, Bugnyar T, 2012. Social bonds and rank acquisition in raven nonbreeder aggregations. Animal behaviour 84:1507-1515. doi: 10.1016/j.anbehav.2012.09.024.
Brent LJN, Heilbronner SR, Horvath JE, Gonzalez-Martinez J, Ruiz-Lambides A, Robinson AG, Skene JHP, Platt ML, 2013. Genetic origins of social networks in rhesus macaques. Scientific Reports 3:1042. doi: 10.1038/srep01042.
Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Machler M, Bolker BM, 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9:378-400.
Brown CR, Brown MB, Shaffer ML, 1991. Food-sharing signals among socially foraging cliff swallows. Animal behaviour 42:551-564.
Brown WM, George M, Wilson AC, 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences 76:1967-1971. doi: 10.1073/pnas.76.4.1967.
Bshary R, Grutter AS, 2005. Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. Biology Letters 1:396-399. doi: 10.1098/rsbl.2005.0344.
Bshary R, Noë R, Biological markets: the ubiquitous influence of partner choice on the dynamics of cleaner fish-client reef fish interactions. Genetic and cultural evolution of cooperation2003. p. 167-184.
Buchan JC, Alberts SC, Silk JB, Altmann J, 2003. True paternal care in a multi-male primate society. Nature 425:179-181. doi: 10.1038/nature01866.

Burnham KP, Anderson DR, 2002. Model Selection and Multimodel Inference. A Practical Information-Theoretic Approach. New York: Springer.
Cairns SJ, Schwager SJ, 1987. A comparison of association indices. Animal behaviour 35:1454-1469. doi: http://dx.doi.org/10.1016/S0003-3472(87)80018-0.
Calenge C, 2011. Home range estimation in R: the adehabitatHR package. Office national de la classe et de la faune sauvage: Saint Benoist, Auffargis, France.
Cameron EZ, Setsaas TH, Linklater WL, 2009. Social bonds between unrelated females increase reproductive success in feral horses. Proceedings of the National Academy of Sciences 106:13850-13853. doi: 10.1073/pnas.0900639106.
Campennì M, Schino G, 2014. Partner choice promotes cooperation: The two faces of testing with agent-based models. Journal of Theoretical Biology 344:49-55. doi: https://doi.org/10.1016/j.jtbi.2013.11.019.
Cant MA, 1998. A model for the evolution of reproductive skew without reproductive suppression. Animal behaviour 55:163-169.
Capitanio JP, Mendoza SP, Lerche NW, Mason WA, 1998. Social stress results in altered glucocorticoid regulation and shorter survival in simian acquired immune deficiency syndrome. Proceedings of the National Academy of Sciences 95:4714-4719. doi: 10.1073/pnas.95.8.4714.
Caro TM, 1990. Cheetah mothers bias parental investment in favour of cooperating sons. Ethology Ecology & Evolution 2:381-395. doi: 10.1080/08927014.1990.9525399.
Caro TM, 1994. Cheetahs of the Serengeti Plains: group living in an asocial species. Chicago, IL, USA: University of Chicago Press.
Carter GG, Farine DR, Wilkinson GS, 2017. Social bet-hedging in vampire bats. Biology Letters 13:20170112.
Carter GG, Schino G, Farine D, 2019. Challenges in assessing the roles of nepotism and reciprocity in cooperation networks. Animal behaviour 150:255-271. doi: https://doi.org/10.1016/j.anbehav.2019.01.006.
Carter GG, Wilkinson GS, 2013. Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. Proceedings of the Royal Society B: Biological Sciences 280:20122573-20122573. doi: 10.1098/rspb.2012.2573.
Chagnon NA, 1988. Life Histories, Blood Revenge, and Warfare in a Tribal Population. Science 239:985-992. doi: 10.1126/science.239.4843.985.
Chapais B, 2002. The role of alliances in social inheritance of rank among female primates. In: Harcourt AH, de Waal FBM, editors. Coalitions and alliances in humans and other animals Oxford, UK: Oxford Science Publications. p. 29-59.
Chapais B, 2006. Kinship, competence and cooperation in primates. In: Kappeler PM, van Schaik CP, editors. Cooperation in Primates and Humans: Mechanisms and Evolution Berlin, Heidelberg: Springer Berlin Heidelberg. p. 47-64.
Chiyo PI, Archie EA, Hollister-Smith JA, Lee PC, Poole JH, Moss CJ, Alberts SC, 2011. Association patterns of African elephants in all-male groups: the role of age and genetic relatedness. Animal behaviour 81:1093-1099. doi: http://dx.doi.org/10.1016/j.anbehav.2011.02.013.
Christiansen F, Sironi M, Moore MJ, Di Martino M, Ricciardi M, Warick HA, Irschick DJ, Gutierrez R, Uhart MM, 2019. Estimating body mass of free-living whales using aerial photogrammetry and 3D volumetrics. Methods in Ecology and Evolution 10:2034-2044. doi: 10.1111/2041-210x.13298.
Clauset A, Newman MEJ, Moore C, 2004. Finding community structure in very large networks. Physical Review E 70:066111.
Clutton-Brock T, 2016. Mammal societies: John Wiley & Sons.
Clutton-Brock TH, 1988. Reproductive success: studies of individual variation in contrasting breeding systems: University of Chicago Press.

Clutton-Brock TH, Albon SD, Gibson RM, Guinness FE, 1979. The logical stag: Adaptive aspects of fighting in red deer (*Cervus elaphus L.*). Animal behaviour 27:211-225. doi: https://doi.org/10.1016/0003-3472(79)90141-6.

Coltman DW, Bancroft DR, Robertson A, Smith JA, Clutton-brock TH, Pemberton JM, 1999. Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. Molecular ecology 8:1199-1209. doi: 10.1046/j.1365-294x.1999.00683.x.

Connor R, Whitehead H, 2005. Alliances II. Rates of encounter during resource utilization: a general model of intrasexual alliance formation in fission–fusion societies. Animal behaviour 69:127-132. doi: https://doi.org/10.1016/j.anbehav.2004.02.022.

Connor RC, 1986. Pseudo-reciprocity: Investing in mutualism. Animal behaviour 34:1562-1566. doi: http://dx.doi.org/10.1016/S0003-3472(86)80225-1.

Connor RC, 1995. The Benefits of Mutualism: A Conceptual Framework. Biological Reviews 70:427-457. doi: 10.1111/j.1469-185X.1995.tb01196.x.

Connor RC, 2010. Cooperation beyond the dyad: on simple models and a complex society. Philos Trans R Soc Lond B Biol Sci 365:2687-2697. doi: 10.1098/rstb.2010.0150.

Connor RC, Cioffi WR, Randić S, Allen SJ, Watson-Capps J, Krützen M, 2017. Male alliance behaviour and mating access varies with habitat in a dolphin social network. Scientific Reports 7:46354. doi: 10.1038/srep46354.

Connor RC, Heithaus MR, Barre LM, 2001. Complex social structure, alliance stability and mating access in a bottlenose dolphin 'super-alliance'. Proceedings of the Royal Society B: Biological Sciences 268:263-267.

Connor RC, Krützen M, 2015. Male dolphin alliances in Shark Bay: changing perspectives in a 30-year study. Animal behaviour 103:223-235. doi: http://dx.doi.org/10.1016/j.anbehav.2015.02.019.

Connor RC, Mann J, 2006. Social cognition in the wild: Machiavellian dolphins. Rational animals:329-367.

Connor RC, Read AJ, Wrangham R, 2000a. Male reproductive strategies and social bonds. In: Mann J, Connor RC, Tyack P, Whitehead H, editors. Cetacean Societies: Field Studies of Dolphins and Whales Chicago, IL, USA: University of Chicago Press. p. 247-269.

Connor RC, Richards AF, Smolker RA, Mann J, 1996. Patterns of Female Attractiveness in Indian Ocean Bottlenose Dolphins. Behaviour 133:37-69.

Connor RC, Smolker R, Bejder L, 2006. Synchrony, social behaviour and alliance affiliation in Indian Ocean bottlenose dolphins, *Tursiops aduncus*. Animal behaviour 72:1371-1378. doi: https://doi.org/10.1016/j.anbehav.2006.03.014.

Connor RC, Smolker RA, Richards AF, 1992. Two levels of alliance formation among male bottlenose dolphins (*Tursiops* sp.). Proceedings of the National Academy of Sciences 89:987-990. doi: 10.1073/pnas.89.3.987.

Connor RC, Watson-Capps JJ, Sherwin WB, Krützen M, 2011. A new level of complexity in the male alliance networks of Indian Ocean bottlenose dolphins (*Tursiops* sp.). Biology Letters 7:623-626. doi: 10.1098/rsbl.2010.0852.

Connor RC, Wells RS, Mann J, Read AJ, 2000b. The bottlenose dolphin: social relationships in a fission-fusion society. In: Mann J, Connor RC, Tyack P, Whitehead H, editors. Cetacean Societies: Field Studies of Dolphins and Whales Chicago, IL, USA: University of Chicago Press. p. 91-126.

Cords M, Thompson NA, 2017. Friendships, coalitions, and alliances. APA handbook of comparative psychology: Basic concepts, methods, neural substrate, and behavior, Vol 1 Washington, DC, US: American Psychological Association. p. 899-913.

Cowlishaw G, 1995. Behavioural Patterns in Baboon Group Encounters: the Role of Resource Competition and Male Reproductive Strategies. Behaviour 132:75. doi: https://doi.org/10.1163/156853995X00298.

Cowlishaw G, Dunbar RIM, 1991. Dominance rank and mating success in male primates. Animal behaviour 41:1045-1056. doi: https://doi.org/10.1016/S0003-3472(05)80642-6.
Crockford C, Wittig RM, Langergraber K, Ziegler TE, Zuberbühler K, Deschner T, 2013. Urinary oxytocin and social bonding in related and unrelated wild chimpanzees. Proceedings of the Royal Society B: Biological Sciences 280:20122765-20122765. doi: 10.1098/rspb.2012.2765.
Crofoot MC, Gilby IC, Wikelski MC, Kays RW, 2008. Interaction location outweighs the competitive advantage of numerical superiority in Cebus capucinus intergroup contests. Proceedings of the National Academy of Sciences 105:577-581.
Crofoot MC, Wrangham RW, 2010. Intergroup Aggression in Primates and Humans: The Case for a Unified Theory. In: Kappeler PM, Silk J, editors. Mind the Gap: Tracing the Origins of Human Universals Berlin, Heidelberg: Springer Berlin Heidelberg. p. 171-195.
Cronin KA, 2012. Prosocial behaviour in animals: the influence of social relationships, communication and rewards. Animal behaviour 84:1085-1093. doi: https://doi.org/10.1016/j.anbehav.2012.08.009.
Csillery K, Johnson T, Beraldi D, Clutton-Brock T, Coltman D, Hansson B, Spong G, Pemberton JM, 2006. Performance of marker-based relatedness estimators in natural populations of outbred vertebrates. Genetics 173:2091-2101. doi: 10.1534/genetics.106.057331.
Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R, Group GPA, 2011. The variant call format and VCFtools. Bioinformatics 27:2156-2158. doi: 10.1093/bioinformatics/btr330.
Darwin C, 1859. The origin of species. London: John Murray.
Dawkins R, 1976. The Selfish Gene. Oxford University Press 1:976.
de Dreu CKW, Greer LL, Handgraaf MJJ, Shalvi S, van Kleef GA, Baas M, Ten Velden FS, van Dijk E, Feith SWW, 2010. The Neuropeptide Oxytocin Regulates Parochial Altruism in Intergroup Conflict Among Humans. Science 328:1408-1411. doi: 10.1126/science.1189047.
de Paoli-Iseppi R, Deagle BE, McMahon CR, Hindell MA, Dickinson JL, Jarman SN, 2017. Measuring Animal Age with DNA Methylation: From Humans to Wild Animals. Frontiers in Genetics 8. doi: 10.3389/fgene.2017.00106.
de Waal FBM, 1984. Sex differences in the formation of coalitions among chimpanzees. Ethology and Sociobiology 5:239-255. doi: https://doi.org/10.1016/0162-3095(84)90004-9.
DeScioli P, Kurzban R, 2009. The alliance hypothesis for human friendship. PLoS One 4:e5802-e5802. doi: 10.1371/journal.pone.0005802.
Dey CJ, Reddon AR, O'Connor CM, Balshine S, 2013. Network structure is related to social conflict in a cooperatively breeding fish. Animal behaviour 85:395-402. doi: https://doi.org/10.1016/j.anbehav.2012.11.012.
Diaz-Aguirre F, Parra GJ, Passadore C, Möller L, 2018. Kinship influences social bonds among male southern Australian bottlenose dolphins (*Tursiops cf. australis*). Behavioral Ecology and Sociobiology 72:190. doi: 10.1007/s00265-018-2621-4.
Díaz-Muñoz SL, Du Val EH, Krakauer AH, Lacey EA, 2014. Cooperating to compete: altruism, sexual selection and causes of male reproductive cooperation. Animal behaviour 88:67-78. doi: http://dx.doi.org/10.1016/j.anbehav.2013.11.008.
Dickerson BR, Brinck K, W., Willson MF, Bentzen P, Quinn TP, 2005. Relative importance of salmon body size and arrival time at breeding grounds to reproductive success. Ecology 86:347-352.
du Val EH, 2007. Adaptive Advantages of Cooperative Courtship for Subordinate Male Lance-Tailed Manakins. The American Naturalist 169:423-432. doi: 10.1086/512137.
Dudley SA, File AL, 2007. Kin recognition in an annual plant. Biology Letters 3:435-438.
Dunbar RIM, 2010. The social role of touch in humans and primates: Behavioural function and neurobiological mechanisms. Neuroscience & Biobehavioral Reviews 34:260-268. doi: https://doi.org/10.1016/j.neubiorev.2008.07.001.

Dunnett CW, 1955. A Multiple Comparison Procedure for Comparing Several Treatments with a Control. Journal of the American Statistical Association 50:1096-1121. doi: 10.1080/01621459.1955.10501294.
Earl DA, von Holdt BM, 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359-361. doi: 10.1007/s12686-011-9548-7.
Ebenau A, von Borell C, Penke L, Ostner J, Schülke O, 2019. Personality homophily affects male social bonding in wild Assamese macaques, *Macaca assamensis*. Animal behaviour 155:21-35. doi: https://doi.org/10.1016/j.anbehav.2019.05.020.
Eisfeld SM, Robinson K, 2004. The Sociality of Bottlenose Dolphins in the Outer Southern Moray Firth, NE Scotland: Implications for Current Management Proposals. European Research on Cetaceans 16:60 - 63.
Elliott E, Ezra-Nevo G, Regev L, Neufeld-Cohen A, Chen A, 2010. Resilience to social stress coincides with functional DNA methylation of the Crf gene in adult mice. Nature Neuroscience 13:1351-1353. doi: 10.1038/nn.2642.
Ellis L, 1995. Dominance and reproductive success among nonhuman animals: A cross-species comparison. Ethology and Sociobiology 16:257-333. doi: https://doi.org/10.1016/0162-3095(95)00050-U.
Engelmann JM, Herrmann E, 2016. Chimpanzees Trust Their Friends. Current Biology 26:252-256. doi: 10.1016/j.cub.2015.11.037.
Ermak J, Brightwell K, Gibson Q, 2017. Multi-level dolphin alliances in northeastern Florida offer comparative insight into pressures shaping alliance formation. Journal of Mammalogy 98:1096-1104. doi: 10.1093/jmammal/gyx053.
Evans KE, Harris S, 2008. Adolescence in male African elephants, *Loxodonta africana*, and the importance of sociality. Animal behaviour 76:779-787. doi: https://doi.org/10.1016/j.anbehav.2008.03.019.
Faaborg J, Parker P, De Lay L, De Vries T, Bednarz J, Paz SM, Naranjo J, Waite T, 1995. Confirmation of cooperative polyandry in the Galapagos hawk (*Buteo galapagoensis*). Behavioral Ecology and Sociobiology 36:83-90.
Farine DR, 2013. Animal social network inference and permutations for ecologists in R using asnipe. Methods in Ecology and Evolution 4:1187-1194.
Fayle TM, Edwards DP, Foster WA, Yusah KM, Turner EC, 2015. An ant–plant by-product mutualism is robust to selective logging of rain forest and conversion to oil palm plantation. Oecologia 178:441-450. doi: 10.1007/s00442-014-3208-z.
Fazioli KL, Hofmann S, Wells RS, 2006. Use of Gulf of Mexico coastal waters by distinct assemblages of bottlenose dolphins (*Tursiops truncatus*). Aquatic Mammals 32:212.
Fiori L, Doshi A, Martinez E, Orams MB, Bollard-Breen B, 2017. The use of unmanned aerial systems in marine mammal research. Remote Sensing 9:543.
Fiori L, Martinez E, Bader MK-F, Orams MB, Bollard B, 2020. Insights into the use of an unmanned aerial vehicle (UAV) to investigate the behavior of humpback whales (*Megaptera novaeangliae*) in Vava'u, Kingdom of Tonga. Marine Mammal Science 36:209-223. doi: 10.1111/mms.12637.
Fisher DO, Lara MC, 1999. Effects of body size and home range on access to mates and paternity in male bridled nailtail wallabies. Animal behaviour 58:121-130. doi: https://doi.org/10.1006/anbe.1999.1119.
Flack Jessica C, de Waal Frans BM, Krakauer David C, 2005. Social Structure, Robustness, and Policing Cost in a Cognitively Sophisticated Species. The American Naturalist 165:E126-E139. doi: 10.1086/429277.
Foerster S, McLellan K, Schroepfer-Walker K, Murray CM, Krupenye C, Gilby IC, Pusey AE, 2015. Social bonds in the dispersing sex: partner preferences among adult female chimpanzees. Animal behaviour 105:139-152. doi: 10.1016/j.anbehav.2015.04.012.

Foroughirad V, Levengood AL, Mann J, Frère CH, 2019. Quality and quantity of genetic relatedness data affect the analysis of social structure. Molecular Ecology Resources 19:1181-1194. doi: 10.1111/1755-0998.13028.
Fowler JH, Dawes CT, Christakis NA, 2009. Model of genetic variation in human social networks. Proceedings of the National Academy of Sciences 106:1720-1724. doi: 10.1073/pnas.0806746106.
Fox J, Weisberg S, 2019. An R Companion to Applied Regression, Third ed. Thousand Oaks, CA, USA: Sage.
Fox KCR, Muthukrishna M, Shultz S, 2017. The social and cultural roots of whale and dolphin brains. Nature Ecology & Evolution 1:1699-1705. doi: 10.1038/s41559-017-0336-y.
Frère CH, Krützen M, Kopps AM, Ward P, Mann J, Sherwin WB, 2010a. Inbreeding tolerance and fitness costs in wild bottlenose dolphins. Proceedings of the Royal Society B: Biological Sciences 277:2667-2673. doi: 10.1098/rspb.2010.0039.
Frère CH, Krützen M, Mann J, Watson-Capps JJ, Tsai YJ, Patterson EM, Connor R, Bejder L, Sherwin WB, 2010b. Home range overlap, matrilineal and biparental kinship drive female associations in bottlenose dolphins. Animal behaviour 80:481-486. doi: http://dx.doi.org/10.1016/j.anbehav.2010.06.007.
Fu F, Nowak MA, Christakis NA, Fowler JH, 2012. The Evolution of Homophily. Scientific Reports 2:845. doi: 10.1038/srep00845.
Gale D, Shapley LS, 1962. College admissions and the stability of marriage. The American Mathematical Monthly 69:9-15.
Gardner A, 2019. The greenbeard effect. Current Biology 29:R430-R431. doi: https://doi.org/10.1016/j.cub.2019.03.063.
Gerber L, Connor RC, King SL, Allen SJ, Wittwer S, Bizzozzero MR, Friedman WR, Kalberer S, Sherwin WB, Wild S, Willems EP, Krützen M, 2020. Affiliation history and age similarity predict alliance formation in adult male bottlenose dolphins. Behavioral Ecology. doi: 10.1093/beheco/arz195.
Ghoul M, Andersen SB, West SA, 2017. Sociomics: Using Omic Approaches to Understand Social Evolution. Trends in Genetics 33:408-419. doi: https://doi.org/10.1016/j.tig.2017.03.009.
Gilby IC, Brent LJN, Wroblewski EE, Rudicell RS, Hahn BH, Goodall J, Pusey AE, 2013. Fitness benefits of coalitionary aggression in male chimpanzees. Behavioral Ecology and Sociobiology 67:373-381. doi: 10.1007/s00265-012-1457-6.
Goldenberg Shifra Z, Douglas-Hamilton I, Wittemyer G, 2016. Vertical Transmission of Social Roles Drives Resilience to Poaching in Elephant Networks. Current Biology 26:75-79. doi: 10.1016/j.cub.2015.11.005.
Gomes CM, Boesch C, 2011. Reciprocity and trades in wild West African chimpanzees. Behavioral Ecology and Sociobiology 65:2183. doi: 10.1007/s00265-011-1227-x.
Graving JM, Chae D, Naik H, Li L, Koger B, Costelloe BR, Couzin ID, 2019. DeepPoseKit, a software toolkit for fast and robust animal pose estimation using deep learning. eLife 8:e47994. doi: 10.7554/eLife.47994.
Greenwood PJ, 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal behaviour 28:1140-1162.
Greminger MP, Stölting KN, Nater A, Goossens B, Arora N, Bruggmann R, Patrignani A, Nussberger B, Sharma R, Kraus RHS, Ambu LN, Singleton I, Chikhi L, van Schaik CP, Krützen M, 2014. Generation of SNP datasets for orangutan population genomics using improved reduced-representation sequencing and direct comparisons of SNP calling algorithms. BMC Genomics 15:16-16. doi: 10.1186/1471-2164-15-16.
Grinnell J, Packer C, Pusey AE, 1995. Cooperation in male lions: kinship, reciprocity or mutualism? Animal behaviour 49:95-105. doi: https://doi.org/10.1016/0003-3472(95)80157-X.

Grueber CE, Nakagawa S, Laws RJ, Jamieson IG, 2011. Multimodel inference in ecology and evolution: challenges and solutions. Journal of Evolutionary Biology 24:699-711. doi: doi:10.1111/j.1420-9101.2010.02210.x.
Grueter CC, Chapais B, Zinner D, 2012. Evolution of Multilevel Social Systems in Nonhuman Primates and Humans. International Journal of Primatology 33:1002-1037. doi: 10.1007/s10764-012-9618-z.
Grueter CC, Qi X, Li B, Li M, 2017. Multilevel societies. Current Biology 27:R984-R986.
Guichoux E, Lagache L, Wagner S, Chaumeil P, Léger P, Lepais O, Lepoittevin C, Malausa T, Revardel E, Salin F, Petit RJ, 2011. Current trends in microsatellite genotyping. Molecular Ecology Resources 11:591-611. doi: 10.1111/j.1755-0998.2011.03014.x.
Hamilton RA, Borcuch T, Allen SJ, Cioffi WR, Bucci V, Krützen M, Connor RC, 2019. Aggression varies with consortship rate and habitat in a dolphin social network. Behavioral Ecology and Sociobiology in press.
Hamilton WD, 1964. The genetical evolution of social behaviour. I. II. Journal of Theoretical Biology:1-52.
Hammerstein P, Noë R, 2016. Biological trade and markets. Philos Trans R Soc Lond B Biol Sci 371. doi: 10.1098/rstb.2015.0101.
Harcourt AH, 1992. Cooperation in Conflicts - Commonalities between Humans and Other Animals. Politics and the Life Sciences 11:251-259.
Harcourt AH, de Waal FBM, 1992. Coalitions and alliances in humans and other animals: Oxford University Press Oxford.
Harrison HB, Saenz-Agudelo P, Planes S, Jones GP, Berumen ML, 2013. Relative accuracy of three common methods of parentage analysis in natural populations. Molecular ecology 22:1158-1170. doi: 10.1111/mec.12138.
Heijne A, Rossi F, Sanfey AG, 2018. Why we stay with our social partners: Neural mechanisms of stay/leave decision-making. Social neuroscience 13:667-679. doi: 10.1080/17470919.2017.1370010.
Heinsohn R, Packer C, 1995. Complex cooperative strategies in group-territorial African lions. Science 269:1260-1262.
Heinsohn RG, 1991. Kidnapping and reciprocity in cooperatively breeding white-winged choughs. Animal behaviour 41:1097-1100. doi: https://doi.org/10.1016/S0003-3472(05)80652-9.
Henzi SP, Barrett L, 2002. Infants as a commodity in a baboon market. Animal behaviour 63:915-921. doi: https://doi.org/10.1006/anbe.2001.1986.
Henzi SP, Lusseau D, Weingrill T, Van Schaik CP, Barrett L, 2009. Cyclicity in the structure of female baboon social networks. Behavioral Ecology and Sociobiology 63:1015-1021.
Hodge SJ, Manica A, Flower TP, Clutton-Brock TH, 2008. Determinants of reproductive success in dominant female meerkats. Journal of Animal Ecology 77:92-102. doi: 10.1111/j.1365-2656.2007.01318.x.
Hoelzel AR, Potter CW, Best PB, 1998. Genetic differentiation between parapatric'nearshore'and'offshore'populations of the bottlenose dolphin. Proceedings of the Royal Society B: Biological Sciences 265:1177.
Holt-Lunstad J, Smith TB, Layton JB, 2010. Social Relationships and Mortality Risk: A Meta-analytic Review. PLOS Medicine 7:e1000316. doi: 10.1371/journal.pmed.1000316.
Horlacher K, 2018. Reproductive success in male bottlenose dolphin alliances in Shark Bay, Western Australia [MSc Thesis]. Zurich: University of Zurich.
Huang Z-Y, Robinson GE, 1996. Regulation of honey bee division of labor by colony age demography. Behavioral Ecology and Sociobiology 39:147-158. doi: 10.1007/s002650050276.
Ilany A, Akçay E, 2016. Social inheritance can explain the structure of animal social networks. Nature Communications 7:12084. doi: 10.1038/ncomms12084.

Jiang Q, Xia D-P, Wang X, Zhang D, Sun B-H, Li J-H, 2019. Interchange between grooming and infant handling in female Tibetan macaques (*Macaca thibetana*). Zoological research 40:139-145. doi: 10.24272/j.issn.2095-8137.2018.049.

Jiang Y, Platt ML, 2018. Oxytocin and vasopressin flatten dominance hierarchy and enhance behavioral synchrony in part via anterior cingulate cortex. Scientific Reports 8:8201. doi: 10.1038/s41598-018-25607-1.

Johnstone RA, 2000. Models of reproductive skew: A review and synthesis. Ethology 106:5-26. doi: 10.1046/j.1439-0310.2000.00529.x.

Kalinowski ST, Taper ML, Marshall TC, 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular ecology 16:1099-1106.

Kappeler PM, 2019. A framework for studying social complexity. Behavioral Ecology and Sociobiology 73:13. doi: 10.1007/s00265-018-2601-8.

Kawanaka K, 1989. Age differences in social interactions of young males in a chimpanzee unit-group at the Mahale Mountains National Park, Tanzania. Primates; journal of primatology 30:285-305. doi: 10.1007/bf02381256.

Kay T, Lehmann L, Keller L, 2019. Kin selection and altruism. Current Biology 29:R438-R442. doi: https://doi.org/10.1016/j.cub.2019.01.067.

Kedzierska KZ, Gerber L, Cagnazzi D, Krützen M, Ratan A, Kistler L, 2018. SONiCS: PCR stutter noise correction in genome-scale microsatellites. Bioinformatics 34:4115-4117. doi: 10.1093/bioinformatics/bty485.

Kellar NM, Catelani KN, Robbins MN, Trego ML, Allen CD, Danil K, Chivers SJ, 2015. Blubber Cortisol: A Potential Tool for Assessing Stress Response in Free-Ranging Dolphins without Effects due to Sampling. PLoS One 10:e0115257. doi: 10.1371/journal.pone.0115257.

Keller BA, Finger J-S, Gruber SH, Abel DC, Guttridge TL, 2017. The effects of familiarity on the social interactions of juvenile lemon sharks, *Negaprion brevirostris*. Journal of Experimental Marine Biology and Ecology 489:24-31. doi: https://doi.org/10.1016/j.jembe.2017.01.004.

Kemper CM, Trentin E, Tomo I, 2014. Sexual maturity in male Indo-Pacific bottlenose dolphins (*Tursiops aduncus*): evidence for regressed/pathological adults. Journal of Mammalogy 95:357-368. doi: 10.1644/13-mamm-a-007.1.

Kerhoas D, Kulik L, Perwitasari-Farajallah D, Agil M, Engelhardt A, Widdig A, 2016. Mother-male bond, but not paternity, influences male-infant affiliation in wild crested macaques. Behavioral Ecology and Sociobiology 70:1117-1130. doi: 10.1007/s00265-016-2116-0.

Killick R, Eckley IA, 2014. changepoint: An R package for changepoint analysis. Journal of Statistical Software 58:1-19.

King SL, Friedman WR, Allen SJ, Gerber L, Jensen FH, Wittwer S, Connor RC, Krützen M, 2018. Bottlenose Dolphins Retain Individual Vocal Labels in Multi-level Alliances. Current Biology 28:1993-1999.e1993. doi: 10.1016/j.cub.2018.05.013.

King SL, Schick RS, Donovan C, Booth CG, Burgman M, Thomas L, Harwood J, 2015. An interim framework for assessing the population consequences of disturbance. Methods in Ecology and Evolution 6:1150-1158. doi: 10.1111/2041-210x.12411.

Klengel T, Pape J, Binder EB, Mehta D, 2014. The role of DNA methylation in stress-related psychiatric disorders. Neuropharmacology 80:115-132. doi: https://doi.org/10.1016/j.neuropharm.2014.01.013.

Knoll AH, 2011. The Multiple Origins of Complex Multicellularity. Annual Review of Earth and Planetary Sciences 39:217-239. doi: 10.1146/annurev.earth.031208.100209.

Kokko H, Johnstone RA, 1999. Social queuing in animal societies: a dynamic model of reproductive skew. Proceedings of the Royal Society B: Biological Sciences 266:571-578. doi: 10.1098/rspb.1999.0674.

Komdeur J, 1996. Influence of age on reproductive performance in the Seychelles warbler. Behavioral Ecology 7:417-425. doi: 10.1093/beheco/7.4.417.

Kopps AM, Ackermann CY, Sherwin WB, Allen SJ, Bejder L, Krützen M, 2014a. Cultural transmission of tool use combined with habitat specializations leads to fine-scale genetic structure in bottlenose dolphins. Proceedings of the Royal Society B: Biological Sciences 281. doi: 10.1098/rspb.2013.3245.

Kopps AM, Krützen M, Allen SJ, Bacher K, Sherwin WB, 2014b. Characterizing the socially transmitted foraging tactic "sponging" by bottlenose dolphins (*Tursiops* sp.) in the western gulf of Shark Bay, Western Australia. Marine Mammal Science 30:847-863.

Kovach AI, Powell RA, 2003. Effects of body size on male mating tactics and paternity in black bears, *Ursus americanus*. Canadian Journal of Zoology 81:1257-1268.

Krakauer AH, 2005. Kin selection and cooperative courtship in wild turkeys. Nature 434:69. doi: 10.1038/nature03325 https://www.nature.com/articles/nature03325#supplementary-information.

Krams I, Krama T, Igaune K, Mänd R, 2008. Experimental evidence of reciprocal altruism in the pied flycatcher. Behavioral Ecology and Sociobiology 62:599-605.

Krützen M, Barré LM, Connor RC, Mann J, Sherwin WB, 2004a. 'O father: where art thou?'—Paternity assessment in an open fission–fusion society of wild bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. Molecular ecology 13:1975-1990. doi: 10.1111/j.1365-294X.2004.02192.x.

Krützen M, Barré LM, Möller LM, Heithaus MR, Simms C, Sherwin WB, 2002. A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. Marine Mammal Science 18:863-878. doi: 10.1111/j.1748-7692.2002.tb01078.x.

Krützen M, Mann J, Heithaus MR, Connor RC, Bejder L, Sherwin WB, 2005. Cultural transmission of tool use in bottlenose dolphins. Proceedings of the National Academy of Sciences 102:8939-8943. doi: 10.1073/pnas.0500232102.

Krützen M, Sherwin WB, Berggren P, Gales N, 2004b. Population structure in an inshore cetacean revealed by microsatellite and mtDNA analysis: bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. Marine Mammal Science 20:28-47. doi: 10.1111/j.1748-7692.2004.tb01139.x.

Krützen M, Sherwin WB, Connor RC, Barré LM, Van de Casteele T, Mann J, Brooks R, 2003. Contrasting relatedness patterns in bottlenose dolphins (*Tursiops* sp.) with different alliance strategies. Proceedings of the Royal Society B: Biological Sciences 270:497-502.

Krützen M, Valsecchi E, Connor R, Sherwin W, 2001. Characterization of microsatellite loci in *Tursiops aduncus*. Molecular Ecology Notes 1:170-172.

Krzyszczyk E, Mann J, 2012. Why become speckled? Ontogeny and function of speckling in Shark Bay bottlenose dolphins (*Tursiops* sp.). Marine Mammal Science 28:295-307. doi: 10.1111/j.1748-7692.2011.00483.x.

Krzyszczyk E, Patterson EM, Stanton MA, Mann J, 2017. The transition to independence: sex differences in social and behavioural development of wild bottlenose dolphins. Animal behaviour 129:43-59. doi: https://doi.org/10.1016/j.anbehav.2017.04.011.

Kuznetsova A, Brockhoff PB, Christensen RHB, 2015. Package 'lmerTest'. R package version 2.

Langergraber K, Mitani J, Vigilant L, 2009. Kinship and social bonds in female chimpanzees (*Pan troglodytes*). American journal of primatology 71:840-851. doi: 10.1002/ajp.20711.

Langergraber KE, Mitani JC, Vigilant L, 2007. The limited impact of kinship on cooperation in wild chimpanzees. Proceedings of the National Academy of Sciences 104:7786-7790. doi: 10.1073/pnas.0611449104.

Langergraber KE, Mitani JC, Watts DP, Vigilant L, 2013. Male–female socio-spatial relationships and reproduction in wild chimpanzees. Behavioral Ecology and Sociobiology 67:861-873. doi: 10.1007/s00265-013-1509-6.

Langmead B, Salzberg SL, 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9:357. doi: 10.1038/nmeth.1923 https://www.nature.com/articles/nmeth.1923#supplementary-information.

Lappin AK, Husak Jerry F, 2005. Weapon Performance, Not Size, Determines Mating Success and Potential Reproductive Output in the Collared Lizard (*Crotaphytus collaris.*). The American Naturalist 166:426-436. doi: 10.1086/432564.
Le Boeuf BJ, 2015. Male-male Competition and Reproductive Success in Elephant Seals. Integrative and Comparative Biology 14:163-176. doi: 10.1093/icb/14.1.163.
Lea AJ, Altmann J, Alberts SC, Tung J, 2016. Resource base influences genome-wide DNA methylation levels in wild baboons (*Papio cynocephalus*). Molecular ecology 25:1681-1696. doi: 10.1111/mec.13436.
Leatherwood S, Reeves RR, 2012. The bottlenose dolphin: Elsevier.
Lemopoulos A, Prokkola JM, Uusi-Heikkilä S, Vasemägi A, Huusko A, Hyvärinen P, Koljonen M-L, Koskiniemi J, Vainikka A, 2019. Comparing RADseq and microsatellites for estimating genetic diversity and relatedness — Implications for brown trout conservation. Ecology and Evolution 9:2106-2120. doi: 10.1002/ece3.4905.
Lewis JS, Wartzok D, Heithaus M, Krützen M, 2013a. Could Relatedness Help Explain Why Individuals Lead in Bottlenose Dolphin Groups? PLoS One 8:e58162. doi: 10.1371/journal.pone.0058162.
Lewis JS, Wartzok D, Heithaus M, Krützen M, 2013b. Could relatedness help explain why individuals lead in bottlenose dolphin groups? PLoS One 8:e58162-e58162. doi: 10.1371/journal.pone.0058162.
Li Z, Rogers E, 2004. Habitat Quality and Activity Budgets of White-Headed Langurs in Fusui, China. International Journal of Primatology 25:41-54. doi: 10.1023/B:IJOP.0000014644.36333.94.
Loretto MC, Fraser ON, Bugnyar T, 2012. Ontogeny of Social Relations and Coalition Formation in Common Ravens (*Corvus corax*). International journal of comparative psychology 25:180-194.
Lukas D, Clutton-Brock T, 2018. Social complexity and kinship in animal societies. Ecology letters 21:1129-1134. doi: doi:10.1111/ele.13079.
Lukas D, Reynolds V, Boesch C, Vigilant L, 2005. To what extent does living in a group mean living with kin? Molecular ecology 14:2181-2196. doi: 10.1111/j.1365-294X.2005.02560.x.
Lusseau D, Schneider K, Boisseau OJ, Haase P, Slooten E, Dawson SM, 2003. The bottlenose dolphin community of Doubtful Sound features a large proportion of long-lasting associations. Behavioral Ecology and Sociobiology 54:396-405. doi: 10.1007/s00265-003-0651-y.
Lynch M, 2007. The Origins of Genome Architecture. Sunderland, Massachusetts, USA: Sinauer Associates.
Macfarlan SJ, Walker RS, Flinn MV, Chagnon NA, 2014. Lethal coalitionary aggression and long-term alliance formation among Yanomamö men. Proceedings of the National Academy of Sciences 111:16662-16669. doi: 10.1073/pnas.1418639111.
Mann J, Connor RC, Barre LM, Heithaus MR, 2000a. Female reproductive success in bottlenose dolphins (*Tursiops* sp.): life history, habitat, provisioning, and group-size effects. Behavioral Ecology 11:210-219.
Mann J, Connor RC, Tyack P, Whitehead H, 2000b. Cetacean societies: field studies of dolphins and whales. Chicago: University of Chicago Press.
Marino L, Connor RC, Fordyce RE, Herman LM, Hof PR, Lefebvre L, Lusseau D, McCowan B, Nimchinsky EA, Pack AA, 2007. Cetaceans have complex brains for complex cognition. PLoS biology 5.
Massen JJM, 2017. Friendships in Animals. In: Vonk J, Shackelford T, editors. Encyclopedia of Animal Cognition and Behavior Cham: Springer International Publishing. p. 1-6.
Massen JJM, Koski SE, 2014. Chimps of a feather sit together: chimpanzee friendships are based on homophily in personality. Evolution and Human Behavior 35:1-8. doi: http://dx.doi.org/10.1016/j.evolhumbehav.2013.08.008.

McCue LM, Cioffi WR, Heithaus MR, Barrè L, Connor RC, 2020. Synchrony, leadership, and association in male Indo-pacific bottlenose dolphins (*Tursiops aduncus*). Ethology n/a. doi: 10.1111/eth.13025.
McDonald DB, 2007. Predicting fate from early connectivity in a social network. Proceedings of the National Academy of Sciences 104:10910-10914. doi: 10.1073/pnas.0701159104.
McDonald DB, Potts WK, 1994. Cooperative display and relatedness among males in a lek-mating bird. Science 266:1030-1032. doi: 10.1126/science.7973654.
McDonald PG, Rollins LA, Godfrey S, 2016. The relative importance of spatial proximity, kin selection and potential 'greenbeard' signals on provisioning behaviour among helpers in a cooperative bird. Behavioral Ecology and Sociobiology 70:133-143. doi: 10.1007/s00265-015-2032-8.
McFarland R, Murphy D, Lusseau D, Henzi SP, Parker JL, Pollet TV, Barrett L, 2017. The 'strength of weak ties' among female baboons: fitness-related benefits of social bonds. Animal behaviour 126:101-106. doi: https://doi.org/10.1016/j.anbehav.2017.02.002.
McPherson M, Smith-Lovin L, Cook JM, 2001. Birds of a Feather: Homophily in Social Networks. Annual Review of Sociology 27:415-444. doi: 10.1146/annurev.soc.27.1.415.
Melis AP, Hare B, Tomasello M, 2006. Chimpanzees Recruit the Best Collaborators. Science 311:1297-1300. doi: 10.1126/science.1123007.
Micheletta J, Waller BM, Panggur MR, Neumann C, Duboscq J, Agil M, Engelhardt A, 2012. Social bonds affect anti-predator behaviour in a tolerant species of macaque, *Macaca nigra*. Proceedings of the Royal Society B: Biological Sciences 279:4042-4050.
Mielke A, Preis A, Samuni L, Gogarten JF, Wittig RM, Crockford C, 2018. Flexible decision-making in grooming partner choice in sooty mangabeys and chimpanzees. Royal Society open science 5. doi: 10.1098/rsos.172143.
Mingramm FMJ, Keeley T, Whitworth DJ, Dunlop RA, 2020. Blubber cortisol levels in humpback whales (*Megaptera novaeangliae*): A measure of physiological stress without effects from sampling. General and Comparative Endocrinology 291:113436. doi: https://doi.org/10.1016/j.ygcen.2020.113436.
Mitani JC, 2009. Male chimpanzees form enduring and equitable social bonds. Animal behaviour 77:633-640. doi: http://dx.doi.org/10.1016/j.anbehav.2008.11.021.
Mitani JC, Merriwether DA, Zhang C, 2000. Male affiliation, cooperation and kinship in wild chimpanzees. Animal behaviour 59:885-893.
Mitani JC, Watts DP, Muller MN, 2002a. Recent developments in the study of wild chimpanzee behavior. Evolutionary Anthropology: Issues, News, and Reviews 11:9-25. doi: 10.1002/evan.10008.
Mitani JC, Watts DP, Pepper JW, Merriwether DA, 2002b. Demographic and social constraints on male chimpanzee behaviour. Animal behaviour 64:727-737. doi: https://doi.org/10.1006/anbe.2002.4014.
Molesti S, Majolo B, 2016. Cooperation in wild Barbary macaques: factors affecting free partner choice. Anim Cogn 19:133-146. doi: 10.1007/s10071-015-0919-4.
Möller LM, Beheregaray LB, 2004. Genetic evidence for sex-biased dispersal in resident bottlenose dolphins (*Tursiops aduncus*). Molecular ecology 13:1607-1612. doi: 10.1111/j.1365-294X.2004.02137.x.
Möller LM, Beheregaray LB, Allen SJ, Harcourt RG, 2006. Association patterns and kinship in female Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) of southeastern Australia. Behavioral Ecology and Sociobiology 61:109-117. doi: 10.1007/s00265-006-0241-x.
Möller LM, Beheregaray LB, Harcourt RG, Krützen M, 2001. Alliance membership and kinship in wild male bottlenose dolphins (*Tursiops aduncus*) of southeastern Australia. Proceedings of the Royal Society B: Biological Sciences 268:1941-1947. doi: 10.1098/rspb.2001.1756.
Möller LM, Harcourt RG, 2008. Shared reproductive state enhances female associations in dolphins. International Journal of Ecology 2008.

Moore BL, Connor RC, Allen SJ, Krützen M, King SL, 2020. Acoustic coordination by allied male dolphins in a cooperative context. Proceedings of the Royal Society B: Biological Sciences 287:20192944.
Mooring MS, Penedo MCT, 2014. Behavioral versus genetic measures of fitness in bison bulls (*Bison bison*). Journal of Mammalogy 95:913-924. doi: 10.1644/13-mamm-a-209.
Mourier J, Vercelloni J, Planes S, 2012. Evidence of social communities in a spatially structured network of a free-ranging shark species. Animal behaviour 83:389-401. doi: http://dx.doi.org/10.1016/j.anbehav.2011.11.008.
Mueller UG, Rehner SA, Schultz TR, 1998. The evolution of agriculture in ants. Science 281:2034-2038.
Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA, 2013. Genotyping-by-sequencing in ecological and conservation genomics. Molecular ecology 22:2841-2847. doi: 10.1111/mec.12350.
Nater A, Kopps AM, Krützen M, 2009. New polymorphic tetranucleotide microsatellites improve scoring accuracy in the bottlenose dolphin *Tursiops aduncus*. Molecular Ecology Resources 9:531-534.
Newton-Fisher NE, Lee PC, 2011. Grooming reciprocity in wild male chimpanzees. Animal behaviour 81:439-446. doi: https://doi.org/10.1016/j.anbehav.2010.11.015.
Nicholson K, Bejder L, Allen SJ, Krützen M, Pollock KH, 2012. Abundance, survival and temporary emigration of bottlenose dolphins (*Tursiops* sp.) off Useless Loop in the western gulf of Shark Bay, Western Australia. Marine and Freshwater Research 63:1059-1068.
Nishida T, Hosaka K, 1996. Coalition strategies among adult male chimpanzees of the Mahale Mountains, Tanzania. Great ape societies:114-134.
Noë R, 1992. Alliance formation among male baboons: shopping for profitable partners. In: Harcourt AH, De Waal FBM, editors. Coalitions and alliances in humans and other animals Oxford, United Kingdom: Oxford University Press.
Noë R, Hammerstein P, 1994. Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behavioral Ecology and Sociobiology 35:1-11. doi: 10.1007/bf00167053.
Noë R, Hammerstein P, 1995. Biological markets. Trends in Ecology & Evolution 10:336-339. doi: https://doi.org/10.1016/S0169-5347(00)89123-5.
Noë R, Sluijter AA, 1995. Which adult male savanna baboons form coalitions? International Journal of Primatology 16:77-105. doi: 10.1007/BF02700154.
Nolin DA, 2011. Kin Preference and Partner Choice. Human Nature 22:156-176. doi: 10.1007/s12110-011-9113-9.
O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, Astashyn A, Badretdin A, Bao Y, Blinkova O, Brover V, Chetvernin V, Choi J, Cox E, Ermolaeva O, Farrell CM, Goldfarb T, Gupta T, Haft D, Hatcher E, Hlavina W, Joardar VS, Kodali VK, Li W, Maglott D, Masterson P, McGarvey KM, Murphy MR, O'Neill K, Pujar S, Rangwala SH, Rausch D, Riddick LD, Schoch C, Shkeda A, Storz SS, Sun H, Thibaud-Nissen F, Tolstoy I, Tully RE, Vatsan AR, Wallin C, Webb D, Wu W, Landrum MJ, Kimchi A, Tatusova T, DiCuccio M, Kitts P, Murphy TD, Pruitt KD, 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Research 44:D733-D745. doi: 10.1093/nar/gkv1189.
Ostner J, Schülke O, 2014. The evolution of social bonds in primate males. Behaviour 151:871. doi: https://doi.org/10.1163/1568539X-00003191.
Owen ECG, Wells RS, Hofmann S, 2002. Ranging and association patterns of paired and unpaired adult male Atlantic bottlenose dolphins, *Tursiops truncatus*, in Sarasota, Florida, provide no evidence for alternative male strategies. Canadian Journal of Zoology 80:2072-2089. doi: 10.1139/z02-195.

Packer C, Gilbert DA, Pusey AE, O'Brieni SJ, 1991. A molecular genetic analysis of kinship and cooperation in African lions. Nature 351:562-565.
Page AE, Chaudhary N, Viguier S, Dyble M, Thompson J, Smith D, Salali GD, Mace R, Migliano AB, 2017. Hunter-Gatherer Social Networks and Reproductive Success. Scientific Reports 7:1153. doi: 10.1038/s41598-017-01310-5.
Parker GA, 1974. Assessment strategy and the evolution of fighting behaviour. Journal of Theoretical Biology 47:223-243.
Parsons KM, Durban JW, Claridge DE, Balcomb KC, Noble LR, Thompson PM, 2003. Kinship as a basis for alliance formation between male bottlenose dolphins, *Tursiops truncatus*, in the Bahamas. Animal behaviour 66:185-194. doi: http://dx.doi.org/10.1006/anbe.2003.2186.
Patzelt A, Kopp GH, Ndao I, Kalbitzer U, Zinner D, Fischer J, 2014. Male tolerance and male–male bonds in a multilevel primate society. Proceedings of the National Academy of Sciences 111:14740-14745. doi: 10.1073/pnas.1405811111.
Peakall R, Smouse PE, 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28:2537-2539. doi: 10.1093/bioinformatics/bts460.
Pemberton JM, Albon SD, Guinness FE, Clutton-Brock TH, Dover GA, 1992. Behavioral estimates of male mating success tested by DNA fingerprinting in a polygynous mammal. Behavioral Ecology 3:66-75. doi: 10.1093/beheco/3.1.66.
Pennisi E, 2005. How Did Cooperative Behavior Evolve? Science 309:93-93. doi: 10.1126/science.309.5731.93.
Penzhorn BL, 1979. Social organisation of the Cape Mountain Zebra *Equus Z. Zebra* in the Mountain Zebra National Park*. Koedoe 22:42. doi: 10.4102/koedoe.v22i1.655.
Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE, 2012. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLoS One 7:e37135. doi: 10.1371/journal.pone.0037135.
Polanowski AM, Robbins J, Chandler D, Jarman SN, 2014. Epigenetic estimation of age in humpback whales. Molecular Ecology Resources 14:976-987. doi: 10.1111/1755-0998.12247.
Pope TR, 1990. The Reproductive Consequences of Male Cooperation in the Red Howler Monkey: Paternity Exclusion in Multi-Male and Single-Male Troops Using Genetic Markers. Behavioral Ecology and Sociobiology 27:439-446.
Poston JP, 1997. Dominance, Access to Colonies, and Queues for Mating Opportunities by Male Boat-Tailed Grackles. Behavioral Ecology and Sociobiology 41:89-98.
Prior NH, Smith E, Dooling RJ, Ball GF, 2019. Familiarity enhances moment-to-moment behavioral coordination in zebra finch (*Taeniopygia guttata*) dyads. Journal of Comparative Psychology:No Pagination Specified-No Pagination Specified. doi: 10.1037/com0000201.
Pritchard JK, Stephens M, Donnelly P, 2000. Inference of Population Structure Using Multilocus Genotype Data. Genetics 155:945-959.
Provençal N, Suderman MJ, Guillemin C, Massart R, Ruggiero A, Wang D, Bennett AJ, Pierre PJ, Friedman DP, Côté SM, Hallett M, Tremblay RE, Suomi SJ, Szyf M, 2012. The Signature of Maternal Rearing in the Methylome in Rhesus Macaque Prefrontal Cortex and T Cells. The Journal of Neuroscience 32:15626-15642. doi: 10.1523/jneurosci.1470-12.2012.
Prugnolle F, de Meeus T, 2002. Inferring sex-biased dispersal from population genetic tools: a review. Heredity 88:161-165. doi: 10.1038/sj.hdy.6800060.
Pryor K, Lindbergh J, 1990. A dolphin-human fishing cooperative in Brazil. Marine Mammal Science 6:77-82. doi: 10.1111/j.1748-7692.1990.tb00228.x.
Pusey AE, 1990. Behavioural Changes At Adolescence in Chimpanzees. Behaviour 115:203-246. doi: doi:https://doi.org/10.1163/156853990X00581.
Qi X-G, Huang K, Fang G, Grueter CC, Dunn DW, Li Y-L, Ji W, Wang X-Y, Wang R-T, Garber PA, Li B-G, 2017. Male cooperation for breeding opportunities contributes to the evolution of multilevel

societies. Proceedings of the Royal Society B: Biological Sciences 284:20171480. doi: 10.1098/rspb.2017.1480.
Queller DC, Ponte E, Bozzaro S, Strassmann JE, 2003. Single-Gene Greenbeard Effects in the Social Amoeba *Dictyostelium discoideum*. Science 299:105. doi: 10.1126/science.1077742.
Randić S, Connor RC, Sherwin WB, Krützen M, 2012. A novel mammalian social structure in Indo-Pacific bottlenose dolphins (*Tursiops* sp.): complex male alliances in an open social network. Proceedings of the Royal Society B: Biological Sciences 279:3083-3090. doi: 10.1098/rspb.2012.0264.
Reeve HK, Emlen ST, Keller L, 1998. Reproductive sharing in animal societies: reproductive incentives or incomplete control by dominant breeders? Behavioral Ecology 9:267-278.
Riley RJ, Gillie ER, Horswill C, Johnstone RA, Boogert NJ, Manica A, 2019. Coping with strangers: how familiarity and active interactions shape group coordination in *Corydoras aeneus*. Royal Society open science 6:190587.
Ripperger SP, Carter GG, Duda N, Koelpin A, Cassens B, Kapitza R, Josic D, Berrío-Martínez J, Page RA, Mayer F, 2019. Vampire Bats that Cooperate in the Lab Maintain Their Social Networks in the Wild. Current Biology 29:4139-4144. doi: https://doi.org/10.1016/j.cub.2019.10.024.
Rodrigues AM, Kokko H, 2016. Models of social evolution: can we do better to predict 'who helps whom to achieve what'? Philos Trans R Soc Lond B Biol Sci 371:20150088.
Romero T, Nagasawa M, Mogi K, Hasegawa T, Kikusui T, 2014. Oxytocin promotes social bonding in dogs. Proceedings of the National Academy of Sciences 111:9085-9090. doi: 10.1073/pnas.1322868111.
Roth TS, Rianti P, Fredriksson GM, Wich SA, Nowak MG, 2020. Grouping behavior of Sumatran orangutans (Pongo abelii) and Tapanuli orangutans (Pongo tapanuliensis) living in forest with low fruit abundance. American journal of primatology 82:e23123. doi: 10.1002/ajp.23123.
Ruckstuhl KE, Neuhaus P, 2001. Behavioral synchrony in ibex groups: effects of age, sex and habitat. 138:1033. doi: https://doi.org/10.1163/156853901753286551.
Ryder TB, Blake JG, Parker PG, Loiselle BA, 2011. The composition, stability, and kinship of reproductive coalitions in a lekking bird. Behavioral Ecology 22:282-290. doi: 10.1093/beheco/arq213.
Sachs JL, Mueller UG, Wilcox TP, Bull JJ, 2004. The evolution of cooperation. The Quarterly Review of Biology 79:135-160.
Samuels A, Gifford T, 1997. A quantitative assessment of dominance relations among bottlenose dolphins. Marine Mammal Science 13:70-99. doi: 10.1111/j.1748-7692.1997.tb00613.x.
Samuni L, Preis A, Mielke A, Deschner T, Wittig RM, Crockford C, 2018. Social bonds facilitate cooperative resource sharing in wild chimpanzees. Proceedings of the Royal Society B: Biological Sciences 285:20181643. doi: 10.1098/rspb.2018.1643.
Sandel AA, Langergraber KE, Mitani JC, 2020. Adolescent male chimpanzees (*Pan troglodytes*) form social bonds with their brothers and others during the transition to adulthood. American journal of primatology 82:e23091. doi: 10.1002/ajp.23091.
Sandel AA, Reddy RB, Mitani JC, 2017. Adolescent male chimpanzees do not form a dominance hierarchy with their peers. Primates; journal of primatology 58:39-49.
Sandell M, 1989. The mating tactics and spacing patterns of solitary carnivores. Carnivore behavior, ecology, and evolution: Springer. p. 164-182.
Say L, Naulty F, Hayden TJ, 2003. Genetic and behavioural estimates of reproductive skew in male fallow deer. Molecular ecology 12:2793-2800. doi: 10.1046/j.1365-294X.2003.01945.x.
Schino G, 2001. Grooming, competition and social rank among female primates: a meta-analysis. Animal behaviour 62:265-271. doi: https://doi.org/10.1006/anbe.2001.1750.
Schino G, Aureli F, 2009a. Chapter 2 Reciprocal Altruism in Primates: Partner Choice, Cognition, and Emotions. Advances in the Study of Behavior: Academic Press. p. 45-69.
Schino G, Aureli F, 2009b. Reciprocal altruism in primates: partner choice, cognition, and emotions. Advances in the Study of Behavior 39:45-69.

Schino G, Aureli F, 2010. A few misunderstandings about reciprocal altruism. Communicative & integrative biology 3:561-563. doi: 10.4161/cib.3.6.12977.
Schino G, Aureli F, 2017. Reciprocity in group-living animals: partner control versus partner choice. Biological Reviews 92:665-672. doi: 10.1111/brv.12248.
Schlötterer C, 2000. Evolutionary dynamics of microsatellite DNA. Chromosoma 109:365-371. doi: 10.1007/s004120000089.
Schoof V, xe, rie AM, Jack KM, Isbell LA, 2009. What Traits Promote Male Parallel Dispersal in Primates? Behaviour 146:701-726.
Schroeder JP, 1990. Breeding bottlenose dolphins in captivity, in "The Bottlenose Dolphin". Academic Press, San Diego, California.
Schülke O, Bhagavatula J, Vigilant L, Ostner J, 2010. Social Bonds Enhance Reproductive Success in Male Macaques. Current Biology 20:2207-2210. doi: http://dx.doi.org/10.1016/j.cub.2010.10.058.
Schulz DJ, Robinson GE, 2001. Octopamine influences division of labor in honey bee colonies. Journal of Comparative Physiology A 187:53-61. doi: 10.1007/s003590000177.
Schweinfurth MK, Neuenschwander J, Engqvist L, Schneeberger K, Rentsch AK, Gygax M, Taborsky M, 2017. Do female Norway rats form social bonds? Behavioral Ecology and Sociobiology 71:98. doi: 10.1007/s00265-017-2324-2.
Schweinfurth MK, Taborsky M, 2018. Relatedness decreases and reciprocity increases cooperation in Norway rats. Proceedings of the Royal Society B: Biological Sciences 285:20180035.
Seyfarth RM, Cheney DL, 2012. The Evolutionary Origins of Friendship. Annual Review of Psychology 63:153-177. doi: 10.1146/annurev-psych-120710-100337.
Sherman PW, 1981. Kinship, Demography, and Belding's Ground Squirrel Nepotism. Behavioral Ecology and Sociobiology 8:251-259.
Sidanius J, Pratto F, 2001. Social dominance: An intergroup theory of social hierarchy and oppression: Cambridge University Press.
Silk J, Cheney D, Seyfarth R, 2013. A practical guide to the study of social relationships. Evolutionary Anthropology: Issues, News, and Reviews 22:213-225. doi: 10.1002/evan.21367.
Silk JB, 2002. Using the'F'-word in primatology. Behaviour 139:421.
Silk JB, 2007a. The adaptive value of sociality in mammalian groups. Philos Trans R Soc Lond B Biol Sci 362:539-559.
Silk JB, 2007b. Social Components of Fitness in Primate Groups. Science 317:1347-1351. doi: 10.1126/science.1140734.
Silk JB, 2009. Nepotistic cooperation in non-human primate groups. Philos Trans R Soc Lond B Biol Sci 364:3243-3254. doi: 10.1098/rstb.2009.0118.
Silk JB, 2014. Evolutionary Perspectives on the Links Between Close Social Bonds, Health, and Fitness. Sociality, hierarchy, health: comparative biodemography: a collection of papers: National Academy of Sciences. p. 121 - 144.
Silk JB, Alberts SC, Altmann J, 2003. Social Bonds of Female Baboons Enhance Infant Survival. Science 302:1231-1234. doi: 10.1126/science.1088580.
Silk JB, Alberts SC, Altmann J, 2006. Social relationships among adult female baboons (*Papio cynocephalus*) II. Variation in the quality and stability of social bonds. Behavioral Ecology and Sociobiology 61:197-204. doi: 10.1007/s00265-006-0250-9.
Silk JB, Alberts SC, Altmann J, Cheney DL, Seyfarth RM, 2012. Stability of partner choice among female baboons. Animal behaviour 83:1511-1518. doi: https://doi.org/10.1016/j.anbehav.2012.03.028.
Silk JB, Beehner JC, Bergman TJ, Crockford C, Engh AL, Moscovice LR, Wittig RM, Seyfarth RM, Cheney DL, 2009. The benefits of social capital: close social bonds among female baboons enhance offspring survival. Proceedings of the Royal Society B: Biological Sciences 276:3099-3104. doi: 10.1098/rspb.2009.0681.

Silk JB, Beehner JC, Bergman TJ, Crockford C, Engh AL, Moscovice LR, Wittig RM, Seyfarth RM, Cheney DL, 2010a. Female chacma baboons form strong, equitable, and enduring social bonds. Behavioral Ecology and Sociobiology 64:1733-1747. doi: 10.1007/s00265-010-0986-0.

Silk JB, Beehner JC, Bergman TJ, Crockford C, Engh AL, Moscovice LR, Wittig RM, Seyfarth RM, Cheney DL, 2010b. Strong and Consistent Social Bonds Enhance the Longevity of Female Baboons. Current Biology 20:1359-1361. doi: http://dx.doi.org/10.1016/j.cub.2010.05.067.

Smith JE, 2014. Hamilton's legacy: kinship, cooperation and social tolerance in mammalian groups. Animal behaviour 92:291-304. doi: http://dx.doi.org/10.1016/j.anbehav.2014.02.029.

Smith JE, Memenis SK, Holekamp KE, 2007. Rank-related partner choice in the fission–fusion society of the spotted hyena (*Crocuta crocuta*). Behavioral Ecology and Sociobiology 61:753-765. doi: 10.1007/s00265-006-0305-y.

Smolker RA, Richards AF, Connor RC, Pepper JW, 1992. Sex Differences in Patterns of Association Among Indian Ocean Bottlenose Dolphins. Behaviour 123:38-69. doi: doi:http://dx.doi.org/10.1163/156853992X00101.

Smuts B, Mann J, 1999. Behavioral development in wild bottlenose dolphin newborns (*Tursiops* sp.). Behaviour 136:529-566.

Spengler FB, Scheele D, Marsh N, Kofferath C, Flach A, Schwarz S, Stoffel-Wagner B, Maier W, Hurlemann R, 2017. Oxytocin facilitates reciprocity in social communication. Social Cognitive and Affective Neuroscience 12:1325-1333. doi: 10.1093/scan/nsx061.

Sprogis KR, Pollock KH, Raudino HC, Allen SJ, Kopps AM, Manlik O, Tyne JA, Bejder L, 2016. Sex-Specific Patterns in Abundance, Temporary Emigration and Survival of Indo-Pacific Bottlenose Dolphins (*Tursiops aduncus*) in Coastal and Estuarine Waters. Frontiers in Marine Science 3. doi: 10.3389/fmars.2016.00012.

St-Pierre A, Larose K, Dubois F, 2009. Long-term social bonds promote cooperation in the iterated Prisoner's Dilemma. Proceedings of the Royal Society B: Biological Sciences 276:4223-4228. doi: 10.1098/rspb.2009.1156.

Städele V, Vigilant L, 2016. Strategies for determining kinship in wild populations using genetic data. Ecology and evolution 6:6107-6120. doi: 10.1002/ece3.2346.

Stanton MA, Gibson QA, Mann J, 2011. When mum's away: a study of mother and calf ego networks during separations in wild bottlenose dolphins (*Tursiops* sp.). Animal behaviour 82:405-412. doi: http://dx.doi.org/10.1016/j.anbehav.2011.05.026.

Staples J, Qiao D, Cho Michael H, Silverman Edwin K, Nickerson Deborah A, Below Jennifer E, 2014. PRIMUS: Rapid Reconstruction of Pedigrees from Genome-wide Estimates of Identity by Descent. The American Journal of Human Genetics 95:553-564. doi: https://doi.org/10.1016/j.ajhg.2014.10.005.

Stone B, Blyde D, Saliki J, Blas-Machado U, Bingham J, Hyatt A, Wang J, Payne J, Crameri S, 2011. Fatal cetacean morbillivirus infection in an Australian offshore bottlenose dolphin (*Tursiops truncatus*). Australian veterinary journal 89:452-457.

Strassmann JE, Page Jr RE, Robinson GE, Seeley TD, 2011. Kin selection and eusociality. Nature 471:E5. doi: 10.1038/nature09833.

Suchak M, Eppley TM, Campbell MW, de Waal FBM, 2014. Ape duos and trios: spontaneous cooperation with free partner choice in chimpanzees. PeerJ 2:e417. doi: 10.7717/peerj.417.

Symonds MRE, Moussalli A, 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behavioral Ecology and Sociobiology 65:13-21. doi: 10.1007/s00265-010-1037-6.

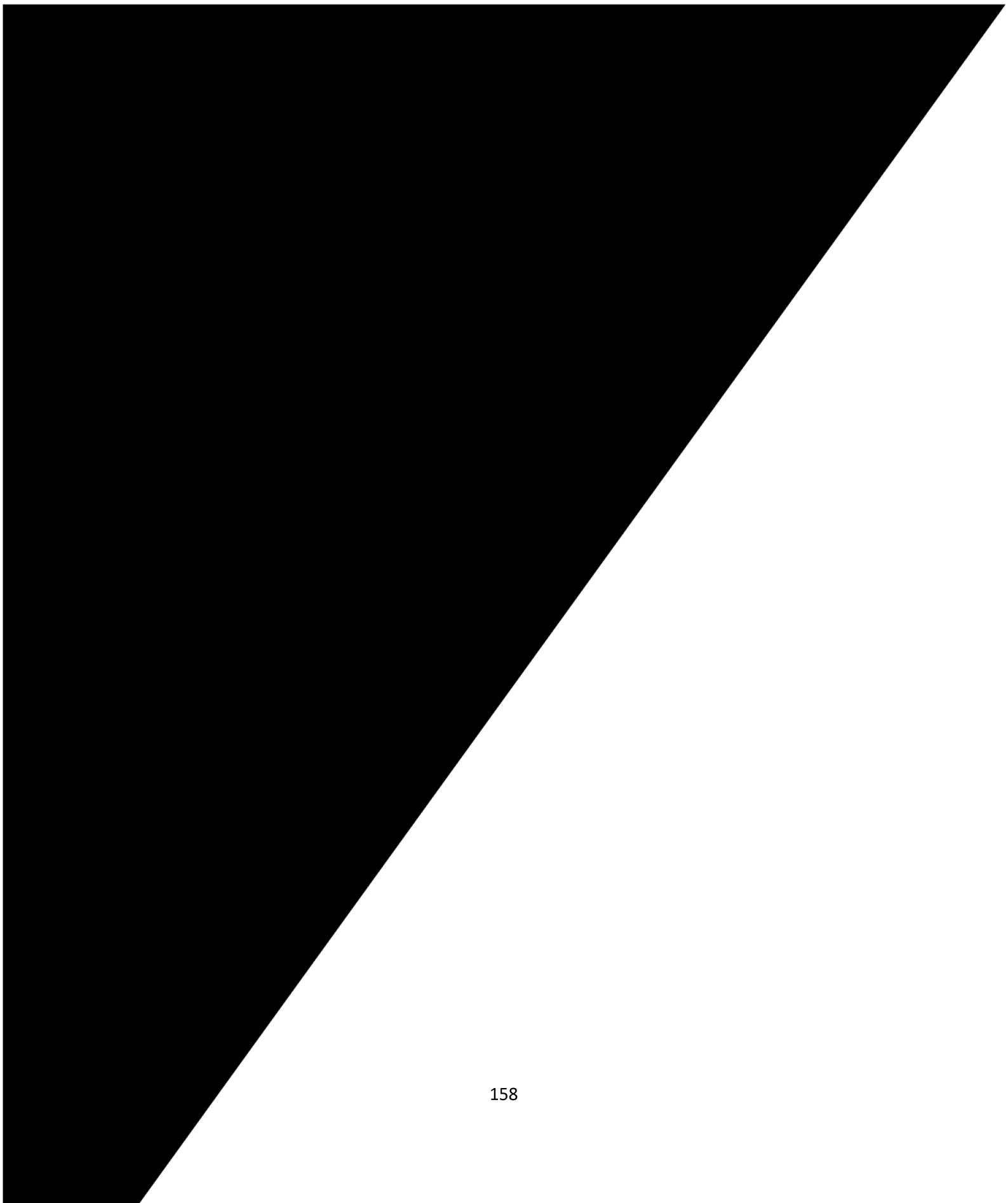
Taborsky M, 1994. Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. Advances in the Study of Behavior 23:1-100.

Taborsky M, Frommen JG, Riehl C, 2016. Correlated pay-offs are key to cooperation. Philos Trans R Soc Lond B Biol Sci 371:20150084.

Taff CC, Campagna L, Vitousek MN, 2019. Genome-wide variation in DNA methylation is associated with stress resilience and plumage brightness in a wild bird. Molecular ecology 28:3722-3737. doi: 10.1111/mec.15186.
Tarr B, Launay J, Dunbar RIM, 2016. Silent disco: dancing in synchrony leads to elevated pain thresholds and social closeness. Evolution and Human Behavior 37:343-349. doi: https://doi.org/10.1016/j.evolhumbehav.2016.02.004.
Tautz D, 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17:6463-6471. doi: 10.1093/nar/17.16.6463.
Taylor HR, 2015. The use and abuse of genetic marker-based estimates of relatedness and inbreeding. Ecology and Evolution 5:3140-3150. doi: 10.1002/ece3.1541.
Author. 2017. R: A Language and Environment for Statistical Computing. Version 3.4.0. Vienna, Austria: R Foundation for Statistical Computing.
Techel F, Zweifel B, Recreational avalanche accidents in Switzerland: trends and patterns with an emphasis on burial, rescue methods and avalanche danger. Proceedings international snow science workshop2013. p. 1106-1112.
Thompson NA, 2019. Understanding the links between social ties and fitness over the life cycle in primates. 156:859. doi: https://doi.org/10.1163/1568539X-00003552.
Thompson NA, Cords M, 2018. Stronger social bonds do not always predict greater longevity in a gregarious primate. Ecology and Evolution 8:1604-1614. doi: 10.1002/ece3.3781.
Thrasher DJ, Butcher BG, Campagna L, Webster MS, Lovette IJ, 2018. Double-digest RAD sequencing outperforms microsatellite loci at assigning paternity and estimating relatedness: A proof of concept in a highly promiscuous bird. Molecular Ecology Resources 18:953-965. doi: 10.1111/1755-0998.12771.
Tiddi B, Aureli F, Schino G, 2012. Grooming up the hierarchy: the exchange of grooming and rank-related benefits in a new world primate. PLoS One 7:e36641-e36641. doi: 10.1371/journal.pone.0036641.
Tin MMY, Rheindt FE, Cros E, Mikheyev AS, 2015. Degenerate adaptor sequences for detecting PCR duplicates in reduced representation sequencing data improve genotype calling accuracy. Molecular Ecology Resources 15:329-336.
Tinbergen N, 1963. On aims and methods of Ethology. Zeitschrift für Tierpsychologie 20:410-433. doi: 10.1111/j.1439-0310.1963.tb01161.x.
Tremblay I, Thomas D, Blondel J, Perret P, Lambrechts MM, 2005. The effect of habitat quality on foraging patterns, provisioning rate and nestling growth in Corsican Blue Tits *Parus caeruleus*. Ibis 147:17-24. doi: 10.1111/j.1474-919x.2004.00312.x.
Trivers R, 1971a. The Evolution of Reciprocal Altruism. The Quarterly Review of Biology 46:35-57.
Trivers R, 1971b. The Evolution of Reciprocal Altruism.
Trivers R, 1972. Parental investment and sexual selection. In: Campbell B, editor. Sexual selection and the descent of man 1871 - 1971 Chicago, IL: Aldine. p. 136-179.
Tsai Y-JJ, Mann J, 2013. Dispersal, philopatry, and the role of fission-fusion dynamics in bottlenose dolphins. Marine Mammal Science 29:261-279. doi: 10.1111/j.1748-7692.2011.00559.x.
Valone TJ, 2007. From eavesdropping on performance to copying the behavior of others: a review of public information use. Behavioral Ecology and Sociobiology 62:1-14.
van Aswegen M, Christiansen F, Symons J, Mann J, Nicholson K, Sprogis K, Bejder L, 2019. Morphological differences between coastal bottlenose dolphin (*Tursiops aduncus*) populations identified using non-invasive stereo-laser photogrammetry. Scientific Reports 9:12235. doi: 10.1038/s41598-019-48419-3.
van Schaik CP, 1999. The socioecology of fission-fusion sociality in Orangutans. Primates; journal of primatology 40:69-86. doi: 10.1007/bf02557703.
Wagner AP, Frank LG, Creel S, 2008. Spatial grouping in behaviourally solitary striped hyaenas, *Hyaena hyaena*. Animal behaviour 75:1131-1142. doi: https://doi.org/10.1016/j.anbehav.2007.08.025.

Wang J, 2007. Triadic IBD coefficients and applications to estimating pairwise relatedness. Genetics Research 89:135-153. doi: doi:10.1017/S0016672307008798.
Wang J, 2011. coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources 11:141-145. doi: 10.1111/j.1755-0998.2010.02885.x.
Wang J, Suri S, Watts DJ, 2012. Cooperation and assortativity with dynamic partner updating. Proceedings of the National Academy of Sciences 109:14363-14368. doi: 10.1073/pnas.1120867109.
Watts DP, 1998. Coalitionary mate guarding by male chimpanzees at Ngogo, Kibale National Park, Uganda. Behavioral Ecology and Sociobiology 44:43-55.
Watts DP, Mitani JC, 2001. Boundary Patrols and Intergroup Encounters in Wild Chimpanzees. Behaviour 138:299-327.
Weidt A, Hofmann SE, König B, 2008. Not only mate choice matters: fitness consequences of social partner choice in female house mice. Animal behaviour 75:801-808. doi: http://dx.doi.org/10.1016/j.anbehav.2007.06.017.
Wells RS, 2014. Social Structure and Life History of Bottlenose Dolphins Near Sarasota Bay, Florida: Insights from Four Decades and Five Generations. In: Yamagiwa J, Karczmarski L, editors. Primates and Cetaceans: Field Research and Conservation of Complex Mammalian Societies Tokyo: Springer Japan. p. 149-172.
West-Eberhard MJ, 1975. The Evolution of Social Behavior by Kin Selection. The Quarterly Review of Biology 50:1-33. doi: 10.1086/408298.
West SA, Ghoul M, 2019. Conflict within cooperation. Current Biology 29:R425-R426. doi: https://doi.org/10.1016/j.cub.2019.04.028.
West SA, Griffin AS, Gardner A, 2007. Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. Journal of Evolutionary Biology 20:415-432. doi: 10.1111/j.1420-9101.2006.01258.x.
White CE, Winans SC, 2007. Cell-cell communication in the plant pathogen *Agrobacterium tumefaciens*. Philos Trans R Soc Lond B Biol Sci 362:1135-1148. doi: 10.1098/rstb.2007.2040.
Whitehead H, 2008. Analyzing Animal Societies. Chicago: University Of Chicago Press.
Whitehead H, 2009. SOCPROG programs: analysing animal social structures. Behavioral Ecology and Sociobiology 63:765-778. doi: 10.1007/s00265-008-0697-y.
Whitehead H, Connor R, 2005. Alliances I. How large should alliances be? Animal behaviour 69:117-126. doi: https://doi.org/10.1016/j.anbehav.2004.02.021.
Widrick AD, 2016. The Function of Second-Order Male Alliances in St. Johns River Dolphins (*Tursiops truncatus*).
Wild S, Allen SJ, Krutzen M, King SL, Gerber L, Hoppitt WJE, 2019a. Multi-network-based diffusion analysis reveals vertical cultural transmission of sponge tool use within dolphin matrilines. Biology Letters 15:20190227. doi: 10.1098/rsbl.2019.0227.
Wild S, Krützen M, Rankin RW, Hoppitt WJE, Gerber L, Allen SJ, 2019b. Long-term decline in survival and reproduction of dolphins following a marine heatwave. Current Biology 29:R239-R240. doi: https://doi.org/10.1016/j.cub.2019.02.047.
Willems EP, Barton RA, Hill RA, 2009. Remotely sensed productivity, regional home range selection, and local range use by an omnivorous primate. Behavioral Ecology 20:985-992.
Willems EP, Hill RA, 2009. Predator-specific landscapes of fear and resource distribution: effects on spatial range use. Ecology 90:546-555.
Wilson DRB, 1995. The ecology of bottlenose dolphins in the Moray Firth, Scotland: a population at the northern extreme of the species' range: University of Aberdeen.
Wiszniewski J, Beheregaray LB, Allen SJ, Möller LM, 2010. Environmental and social influences on the genetic structure of bottlenose dolphins (*Tursiops aduncus*) in Southeastern Australia. Conservation Genetics 11:1405-1419. doi: 10.1007/s10592-009-9968-z.

Wiszniewski J, Brown C, Möller LM, 2012a. Complex patterns of male alliance formation in a dolphin social network. Journal of Mammalogy 93:239-250. doi: 10.1644/10-mamm-a-366.1.
Wiszniewski J, Corrigan S, Beheregaray LB, Möller LM, 2012b. Male reproductive success increases with alliance size in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*). Journal of Animal Ecology 81:423-431. doi: doi:10.1111/j.1365-2656.2011.01910.x.
Wittemyer G, Douglas-Hamilton I, Getz WM, 2005. The socioecology of elephants: analysis of the processes creating multitiered social structures. Animal behaviour 69:1357-1371.
Wroblewski EE, Murray CM, Keele BF, Schumacher-Stankey JC, Hahn BH, Pusey AE, 2009. Male dominance rank and reproductive success in chimpanzees, *Pan troglodytes schweinfurthii*. Animal behaviour 77:873-885. doi: 10.1016/j.anbehav.2008.12.014.
Würsig B, Würsig M, 1977. The Photographic Determination of Group Size, Composition, and Stability of Coastal Porpoises (*Tursiops truncatus*). Science 198:755-756. doi: 10.1126/science.198.4318.755.
Young C, Majolo B, Heistermann M, Schülke O, Ostner J, 2014a. Responses to social and environmental stress are attenuated by strong male bonds in wild macaques. Proceedings of the National Academy of Sciences 111:18195-18200. doi: 10.1073/pnas.1411450111.
Young C, Majolo B, Schülke O, Ostner J, 2014b. Male social bonds and rank predict supporter selection in cooperative aggression in wild Barbary macaques. Animal behaviour 95:23-32. doi: https://doi.org/10.1016/j.anbehav.2014.06.007.
Zannas AS, West AE, 2014. Epigenetics and the regulation of stress vulnerability and resilience. Neuroscience 264:157-170. doi: https://doi.org/10.1016/j.neuroscience.2013.12.003.
Zuberbühler K, 2008. Audience effects. Current Biology 18:R189-R190.
Zuur AF, Ieno EN, Smith GM, 2007. Analyzing ecological data. New York, New York, USA: Springer Science & Business Media.
Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM, 2009. Mixed effects models and extensions in ecology with R. New York, New York, USA: Springer.



7. Co-authored Publications and Reprints of Publications

Wild S., Allen S.J., Krützen M., King S.L., **Gerber L.**, Hoppitt W.J.E.: Multi-network-based diffusion analysis reveals vertical cultural transmission of sponge tool use within dolphin matrilines. Biology Letters (2019)
https://doi.org/10.1098/rsbl.2019.0227

Bizzozzero M.R., Allen S.J., **Gerber L.**, Wild S., King S.L., Connor R.C.C., Friedman W.R., Wittwer S., Krützen M.: Tool use and social homophily among male bottlenose dolphins. Proceedings of the Royal Society B: Biological Sciences (2019)
https://doi.org/10.1098/rspb.2019.0898

Wild S., Krützen M., Rankin R.W., Hoppitt W.J.E., **Gerber L.**, Allen S.J.: Long-term decline in survival and reproduction of dolphins following a marine heatwave. Current Biology (2019)
https://doi.org/10.1016/j.cub.2019.02.047

Kedzierska K.Z., **Gerber L.**, Krützen M., Ratan A., Kistler L.,: SONiCS: PCR stutter noise correction in genome-scale microsatellites. Bioinformatics (2018)
https://doi.org/10.1093/bioinformatics/bty485

King S.L., Friedman W.R., Allen S.J., **Gerber L.**, Jensen F.H., Wittwer S., Connor R.C.C., Krützen M.: Bottlenose Dolphins Retain Individual Vocal Labels in Multi-level Alliances. Current Biology (2018)
https://doi.org/10.1016/j.cub.2018.05.013

Allen S.J., Bryant K.A., Kraus R.H., Loneragan N.R., Kopps A.M., Brown A.M., **Gerber L.**, Krützen M.: Genetic isolation between coastal and fishery-impacted, offshore bottlenose dolphin (*Tursiops* spp.) populations. Molecular Ecology (2016)
https://doi.org/10.1111/mec.13622

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Original Article

Affiliation history and age similarity predict alliance formation in adult male bottlenose dolphins

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Male alliances are an intriguing phenomenon in the context of reproduction since, in most taxa, males compete over an indivisible resource, female fertilization. Adult male bottlenose dolphins (*Tursiops aduncus*) in Shark Bay, Western Australia, form long-term, multilevel alliances to sequester estrus females. These alliances are therefore critical to male reproductive success. Yet, the long-term processes leading to the formation of such complex social bonds are still poorly understood. To identify the criteria by which male dolphins form social bonds with other males, we adopted a long-term approach by investigating the ontogeny of alliance formation. We followed the individual careers of 59 males for 14 years while they transitioned from adolescence (8–14 years of age) to adulthood (15–21 years old). Analyzing their genetic relationships and social associations in both age groups, we found that the vast majority of social bonds present in adolescence persisted through time. Male associations in early life predict alliance partners as adults. Kinship patterns explained associations during adolescence but not during adulthood. Instead, adult males associated with males of similar age. Our findings suggest that social bonds among peers, rather than kinship, play a central role in the development of adult male polyadic cooperation in dolphins.

Lay Summary: Multilevel cooperation in adult male bottlenose dolphins is based on friendships that are formed among similarly aged males during their adolescence. Although cooperative behaviors in many animals are found among relatives, this is not the case in dolphins. Our findings reveal the existence of enduring friendships in a complex marine mammal society, similar to those that have been described in many primate species including humans.

Key words: **bottlenose dolphin, cooperation, kin selection, male alliance, multilevel society, social bonds.**

INTRODUCTION

Social animals frequently interact with their group members. Although such interactions can occur among different sets of individuals (e.g., Henzi et al. 2009; Braun and Bugnyar 2012; Schweinfurth et al. 2017), some individuals interact repeatedly in beneficial contexts and over a longer period of time (Schino and Aureli 2017). In many studies on wild animal populations, such long-term stable interactions among the same individuals have previously been referred to as "social bonds" (Massen 2017), defined as persistent,

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affiliative, and cooperative relationships that may generate benefits through support which may be approximately balanced between the partners (Silk 2002; Ostner and Schülke 2014). It has previously been shown that "social bonds" involve some degree of differentiation and selectivity in that individuals maintaining these bonds showed high rates of association (Silk 2002; Whitehead 2008; Ostner and Schülke 2014; Thompson 2019). Here, we follow this convention by referring to two individuals as having a social bond whenever they show significantly higher association rates than the group average over an extended period of time (Whitehead 2008; Ostner and Schülke 2014).

Benefits of social bonds include increased reproductive output (e.g., feral horses *Equus ferus*, Cameron et al. 2009; chimpanzees *Pan troglodytes*, Langergraber et al. 2013; Assamese macaques *Macaca assamensis*, Schülke et al. 2010; house mice *Mus musculus*, Weidt et al. 2008), life span (e.g., savannah baboons *Papio cynocephalus*, Silk et al. 2010), infant survival (e.g., savannah baboons, Silk et al. 2003, 2009), and decreased mortality (e.g., humans, Holt-Lunstad et al. 2010). Social bonds are thus crucial determinants of an individual's fitness. Fitting with predictions of kin selection theory (Hamilton 1964), relatives are often preferred social partners, with closer kin being preferred over distant kin (see Smith 2014 for a review).

Philopatry facilitates the formation of persistent social bonds and permits close association with kin postweaning. Most mammals are characterized by female philopatry and male dispersal (Greenwood 1980), allowing females to form close and enduring social bonds. This sex-bias can be explained by the different reproductive constraints faced by both sexes. Although reproductive success of females is limited by access to resources and safety, male reproductive success is constrained by access to fertile females (Trivers 1972).

Due to the indivisibility of fertilizations, a paradox exists where males cooperate to reproduce (Díaz-Muñoz et al. 2014). Males often engage in temporary coalitions, in which 2 or more males cooperate to compete against others in contests over resources or rank (Smith 2014), thereby increasing individual access to females. Recurring coalitions among the same males, also known as male alliances (Harcourt 1992), are an unusual phenomenon (Díaz-Muñoz et al. 2014). Alliances occur in, though are not limited to, humans (Chagnon 1988), nonhuman primates (e.g., chimpanzees, Mitani et al. 2002a; savannah baboons, Noë 1992), lions (*Panthera leo*, Packer et al. 1991), cheetahs (*Acinonyx jubatus*, Caro 1994), lekking birds (e.g., manakins *Chiroxiphia linearis*, McDonald and Potts 1994), fish (reviewed in Taborsky 1994), and bottlenose dolphins (*Tursiops aduncus*, Connor et al. 1992).

Kin selection theory can resolve the apparent paradox of persisting social bonds between allied males when individuals are related (Caro 1990; Pope 1990; Qi et al. 2017). Polytocous species, those that give birth to multiple offspring at once, may produce "ready-made" male alliances from single litters (Caro 1990) or synchronized litters of related individuals (Packer et al. 1991). In contrast, many monotocous species, which produce single offspring, share the demographic challenge of maternal same-sex half-siblings being scarce and, if present, separated in age by at least several years. In chimpanzees, for instance, Langergraber et al. (2007) found that, although males do favor maternal siblings as allies, the majority of cooperative male dyads were formed among nonrelatives.

In some species, for example, long-tailed manakins and boat-tailed grackles (McDonald and Potts 1994; *Quiscalus major*, Poston 1997), nonkin male cooperation can be attributed to social queuing, where subordinates remain in a group to acquire dominant status (Kokko and Johnstone 1999). However, neither kin selection nor social queuing can explain the persisting social bonds formed among unrelated males observed in, for example, humans (Boehm 2009), baboons (Guinea baboons *Papio papio*, Patzelt et al. 2014), chimpanzees (Watts 1998), Galapagos hawks (*Buteo galapagoensis*, Faaborg et al. 1995), and dolphins (Wiszniewski et al. 2012b; Connor and Krützen 2015). To understand how persisting social bonds among unrelated males emerge, we need to investigate how, when, and with whom such bonds are formed.

Male Indo-Pacific bottlenose dolphins in Shark Bay, Western Australia, exhibit one of the most remarkable male alliance systems known (Connor and Krützen 2015). The system is unusual because male dolphins exhibit long-term, multilevel alliances with context-dependent interactions within an open social network (Connor et al. 2000a, 2017). This sets them apart from the multilevel alliances observed in baboon species (Grueter et al. 2012). Although male dolphins in other populations form alliances (Owen et al. 2002; Parsons et al. 2003; Wiszniewski et al. 2012a), Shark Bay appears to be the only population where up to 3 levels of nested alliances occur (Randić et al. 2012; Connor and Krützen 2015).

Alliance membership provides male dolphins with mating opportunities they would otherwise not obtain, as nonallied males have been shown to sire very few, if any, offspring (Krützen et al. 2004a). On the level of first-order alliances, 2 to 3 males cooperate to coerce individual estrus females. Members of first-order alliances belong to larger groups of second-order alliances, consisting of 4–14 males, which compete against other such alliances over access to females (Connor et al. 1992, 2011). Males select their first-order allies from within their second-order alliance (Connor et al. 2011). Thus, second-order alliances are now recognized as the core social unit of male dolphins in Shark Bay (Connor and Krützen 2015). Once formed in early adulthood, second-order alliances can persist over decades (Connor and Krützen 2015). Adult male dolphins are characterized by high levels of association year-round (Smolker et al. 1992), increased rates of affiliative behaviors such as petting (Connor et al. 2000a, 2006) and high levels of cooperation during consortships (Connor and Krützen 2015). Male alliances in dolphins therefore differ from the purely reproductive alliances or coalitions observed in other species (Silk 2002; Díaz-Muñoz et al. 2014; Thompson 2019). Male alliance partners in dolphins can therefore be said to exhibit social bonds based on the definition used in this study.

Preliminary work on relatedness among males in Shark Bay was ambiguous in that relatedness patterns differed markedly between alliances of different sizes and levels of stability (Krützen et al. 2003). However, Krützen et al. (2003) and studies on other dolphin populations (Möller et al. 2001; Parsons et al. 2003; Wiszniewski et al. 2012a; Diaz-Aguirre et al. 2018) assessed the potential effect of kinship based on average group relatedness within established alliances relative to a population mean. In order to reveal the development of male alliance formation, however, individual males and their social bonds with others during alliance development need to be considered. Furthermore, the individual-based approach we employed here has an additional advantage in that it accounts for the availability of an individual's potential associates, unlike earlier studies assuming equal availability of potential associates.

Previous work demonstrated that male calves and juveniles (2 years pre-weaning and 2 years post-weaning, respectively) invest more time into developing social skills compared with females of the same age (Stanton et al. 2011; Krzyszczyk et al. 2017). The persistence of specific dyadic associations, though, has never been tested. Adolescence is a critical period during which males become sexually and socially mature (Connor and Krützen 2015). In contrast to adult

males, adolescent males have not yet established alliance membership and do not engage in consortships. Thus, patterns of associations are expected to differ qualitatively and quantitatively between adolescent and adult males. To close this important gap in our understanding of alliance formation, we explored the development and maintenance of social bonds during the transition from adolescence to adulthood.

Here, we describe the ontogeny of social bonds and thus, alliance formation among male bottlenose dolphins relative to patterns of genetic relatedness, affiliation history, and age-similarity. We focus on social bonds of individual males during the transition from adolescence to adulthood. This approach permitted us to gain insights into the underlying dynamics of friendships among male bottlenose dolphins.

MATERIALS AND METHODS

Data collection and study subjects

Data were collected at our long-term field site in the eastern gulf of Shark Bay, Western Australia. Standardized observations, hereafter "surveys," have been conducted on this Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) population since 1984 (Connor and Krützen 2015). In this study, we extracted survey data on group composition recorded between the years 1988 and 2015. Group composition was determined during the first 5 min of each survey according to the 10-m "chain rule" using photographs of dorsal fins to identify individuals (Smolker et al. 1992).

We extracted association data on 59 focal males, each of which we followed over 14 years. Data were extracted for 2 periods in the lifespan of each focal male, creating 2 age groups: from 8 to 14 years ("adolescence") and from 15 to 21 years old ("adulthood"). By age 8, male bottlenose dolphins have typically been weaned for approximately 4 years and have transitioned to being independent juveniles (Mann et al. 2000a). Male bottlenose dolphins reach sexual maturity between 12 and 15 years of age (Kemper et al. 2014) and start forming stable alliances at approximately 15 years of age (Connor et al. 2000a). Only alliance membership enables males to successfully consort females (Connor et al. 1992). Males under 13 years of age have never been observed consorting an adult female, although this does not exclude the possibility that some males might start at a younger age (Connor and Krützen 2015). To avoid biases introduced by unbalanced age ranges, we limited the upper age in the adult category to 21 years of age, although dolphins may live past 40 in this population (unpublished data).

Whenever possible, birth dates were assigned based on the first sighting of a dolphin as a calf. Calf age was estimated using behavioral and physical criteria, including surfacing patterns, presence of fetal folds, as well as the last sighting of the mother before birth (Smuts and Mann 1999). In cases where there were no early-life demographic data available, we used ventral speckle density to infer approximate birth date, which is highly correlated with age in our study population (Krzyszczyk and Mann 2012).

Association rates

Socio-positive behaviors such as grooming in primates (Dunbar 2010) are often used to infer social bond strength on the qualitative level. However, social bonds can also be identified quantitatively by increased rates of affiliation over a prolonged period of time compared with other relationships in the group (Silk 2002; Whitehead 2008; Ostner and Schülke 2014; Thompson 2019). Since socio-positive behaviors cannot be observed systematically underwater, we used association rates to identify social bonds among male dolphins. Association rates are well-established indicators for social bond strength in cetaceans (Mann et al. 2000b) and have been demonstrated to correlate with social bond strength (Silk 2002; Mitani 2009). To quantify associations between focal males and their male associates, we calculated Half-Weight Association Indices (HWIs, Cairns and Schwager 1987) for each focal male twice: once during its adolescence and for a second time while adult. A focal male's associates consisted of all males with whom a focal was observed. Both focal males and all their associates had to have been observed at least 30 times in each age group. We used SOCPROG V2.6 (Whitehead 2009) to generate HWIs with the sampling period set to 1 day. Group composition data were collected during surveys, and re-sights, that is, the same group encountered within 2 h, were excluded.

To identify social bonds among focal males and their associates in each age group, we conducted a change point analysis. Change points are those where statistical properties of the data to either side differ, as is expected for different levels of social structures in multilevel societies (e.g., Wittemyer et al. 2005; Patzelt et al. 2014). To locate change points, we used the "changepoint" (Killick and Eckley 2014) R package employing the Pruned Exact Linear Time (PELT) method. The data set for the change point analysis comprised all dyads associating during the focal male's adulthood, denoted by a HWI larger than 0 ($N = 1{,}755$). Adulthood HWIs were higher (mean ± sd = 0.12 ± 0.18, $N = 1{,}755$) than those in adolescence (mean ± sd = 0.10 ± 0.13, $N = 1{,}544$). Including adulthood HWIs only resulted in a more conservative cutoff for the adolescence period while reliably identifying a focal male's alliance partner once adulthood was reached.

Based on the changepoint analysis, we defined a cutoff HWI value above which we regarded 2 males as sharing a social bond due to association rates well-above random expectations (Silk 2002; Whitehead 2008; Ostner and Schülke 2014). For focal adolescent males and any other male above the cutoff value, we chose the term "close associates." Correspondingly, for focal adult males and any other male above the cutoff, we used "alliance partners." Exploring the dyadic nature of male bonds in 2 separate age periods allowed us to determine some of the proximate mechanisms that predict alliance formation in adult male dolphins for the first time.

Pairwise relatedness and mitochondrial matriline membership

To explore the potential role of kinship in male association patterns, we used both maternal mitochondrial DNA (mtDNA) haplotypes and pairwise autosomal relatedness estimates. DNA was extracted from tissue samples which were collected using a remote biopsy system for small cetaceans (Krützen et al. 2002). To assign individuals to their maternal haplotype, we sequenced a 468 base-pair fragment of the hypervariable region I of mtDNA, as described in Bacher et al. (2010). We estimated pairwise relatedness based on 22 hypervariable autosomal microsatellite markers, which we generated and scored using previously established protocols (Kopps et al. 2014). We provide summary statistics on genetic diversity indices in Supplementary Material, see Tables S2–S4 and Figures S1 and S2.

Relatedness estimators perform differently based on the inherent relatedness composition of a population (Csillery et al. 2006). We identified the best performing relatedness estimator (i.e., high precision, low standard deviation) for our population by choosing the

estimator with the highest correlation between simulated relatedness estimates and true relatedness values, that is, the values expected for a certain relationship, using the software Coancestry V1.0.1.5 (Wang 2011). We included all individuals with no more than 3 missing loci and simulated 1,000 relatedness values based on the allele frequency distribution in our population. Simulations were completed for each of the following relationships: parent–offspring (PO), half-siblings (HS), and unrelated individuals (UR). We then compared the correlations between the simulated values and the expected true relatedness values (PO = 0.5, HS = 0.25, UR = 0) for all estimators. The triadic likelihood estimator TrioML (Wang 2007) showed the highest correlation and was therefore our relatedness estimator of choice.

Male–male associations during adolescence and adulthood

We quantified the effect of shared mtDNA haplotype, pairwise relatedness, age, and association rates during adolescence on adulthood association rates by building 2 linear mixed models. For both models, the response variables were the HWIs between focal males and their close associates during adolescence, and their alliance partners in adulthood, respectively. Advancing upon previous research in this area (Möller et al. 2001; Krützen et al. 2003; Parsons et al. 2003; Wiszniewski et al. 2012a; Diaz-Aguirre et al. 2018), this approach allowed us to analyze the ontogeny of alliance formation on an individual level during this critical period.

For the adolescence model (Model 1), the explanatory variables we tested were age difference in years, pairwise relatedness, and the presence or absence of a shared mtDNA haplotype. In the adulthood model (Model 2), we investigated association patterns of the focal males during their adulthood and included the same variables as Model 1, as well as a dyad's HWI during the focal male's adolescence. This additional variable permitted us to test whether a dyad's association during the focal male's adolescence predicts their association once the focal male reached adulthood. To control for repeated measures of individuals, we included the ID code of the focal male and his close associates (Model 1) or alliance partners (Model 2) as random effects. All analyses were carried out in R (R Core Team 2017), V3.4.0 and linear mixed models were built using the R package "lme4" (Bates et al. 2015).

To identify which combination of the above variables best described the observed association patterns during adolescence and adulthood, we first calculated a global model including all variables (Supplementary Table S1: Models 1 and 2). Subsequently, we used the "dredge" function in MuMIn (Barton and Barton 2018) to obtain second-order Akaike's Information Criterion values corrected for small sample size (AICcs) for models containing only a subset of variables. Differences in AICc (ΔAICc) allowed us to determine the best models among candidate solutions (Zuur et al. 2007). We then averaged across the top model set separately for the adolescence (Model 1) and the adulthood model (Model 2), where ΔAICc < 2 (Burnham and Anderson 2003; Grueber et al. 2011). All models with ΔAICc < 2 are shown in Table 1.

Within-dyad relationship development

We wanted to determine whether age, relatedness, or shared mtDNA haplotype influenced the formation, maintenance, or termination of social bonds between adolescence and adulthood. For this, we combined data from the previous analyses to classify each dyad in one of the following 3 categories of within-dyad relationship development ("RD"): close associates during adolescence and alliance partners in adulthood ("ASA"); close associates during adolescence but not alliance partners in adulthood ("ASO"); alliance partners in adulthood without having been close associates during adolescence ("AO").

We built a total of 9 binomial generalized linear mixed models (Supplementary Table S1: Models 4a–c), in which we entered either pairwise relatedness, shared mtDNA haplotype or age difference as explanatory variables, and investigated their effect on the 3 relationship development scenarios. The variables were tested separately because models including combinations of the explanatory variables did not converge. To account for multiple tests, we adjusted the significance level to 0.0056 (Bonferroni correction, Dunnett 1955). In models (a), we were interested in whether males who became alliance partners without having been close associates (AO) differed in either age, relatedness, or shared haplotype from males that became alliance partners and were close associates during adolescence (ASA). In models (b), we investigated if any of the 3 variables were correlated with whether close associates during adolescence became alliance partners once adulthood was reached (ASA) or not (ASO). Lastly, in models (c), we tested whether male dyads with social bonds only during either adolescence or adulthood, that is, either being close associates (ASO) or alliance partners (AO) only, differed with respect to age difference, relatedness, or haplotype similarity.

Statistical significance of fixed effects was assessed using Satterthwaite's method as implemented in the package "lmertest" (Kuznetsova et al. 2017). All models presented above were validated by likelihood ratio tests where we compared each model to a null model containing only the intercept and random effects. All models performed significantly better than their respective null models. To test for multicollinearity among our explanatory variables, we calculated the Variance Inflation Factors (VIFs), running the script containing the "corvif" function provided by Zuur (2009). None of our variables were correlated (Supplementary Table S5). Lastly, we checked the model assumptions visually by Q-Q plots, as well as histograms of the residuals (provided in Supplementary Material). Interactions were not significant in all models and were, thus, not incorporated.

RESULTS

Identification of "close associates" and "alliance partners"

We identified 3 change points (Figure 1) reflecting the multilevel social structure of male associations in our population. The first change point, observed at HWI of ≥ 0.38, separates first-order alliance partners from second-order alliance partners (N = 201, mean = 0.55). The second change point, at HWI ≥ 0.19, divides second-order alliance partners from males that occasionally associate (N = 160, mean = 0.28). The third change point, at HWI ≥ 0.09, separates males that occasionally associate (N = 182, mean = 0.12) from those that randomly associate (N = 1,212, mean = 0.03). We treated all dyads with a HWI ≥ 0.19 (i.e., the second change point detected) as close associates during adolescence. The same value was used to identify alliance partners once the focal males transitioned into adulthood. This value is supported by earlier studies on the same population, showing that a HWI ≥ 0.2 validly clustered males into their respective second-order alliances (Smolker et al. 1992; Connor et al. 2001; King et al. 2018).

Table 1

Models with ΔAICc < 2 compared with the top models

	Model	AICc	Δ AICc	Weight
1a	$HWI_{adolescence} \sim r + \Delta age + (1 \mid ID_F) + (1 \mid ID_A)$	−304.72	0	0.42
1b	$HWI_{adolescence} \sim r + (1 \mid ID_F) + (1 \mid ID_A)$	−304.54	0.18	0.39
1c	$HWI_{adolescence} \sim r + \Delta age + haplotype + (1 \mid ID_F) + (1 \mid ID_A)$	−303.11	1.6	0.19
2a	$HWI_{adulthood} \sim HWI_{adolescence} + \Delta age + (1 \mid ID_F) + (1 \mid ID_A)$	−448.1	0	0.63
2b	$HWI_{adulthood} \sim HWI_{adolescence} + haplotype + \Delta age + (1 \mid ID_F) + (1 \mid ID_A)$	−446.99	1.1	0.37

Results of model selection including model details of all models with ΔAICc < 2. Models 1a–c were averaged to identify the effect of relatedness (r), shared haplotype, and age difference (Δage) on associations during adolescence. Individual ID codes are included as random effects (ID_F = ID focal, ID_A = ID Close Associate). Models 2a–b were averaged to explore how associations during adolescence, age difference, and shared haplotype influence adulthood association patterns. For the adulthood models, ID_A denotes alliance partners but all other abbreviations are consistent with the ones described for Models 1a–c. Relatedness was not included in the best adult models. Weight describes the proportion of a given model to the averaged model.

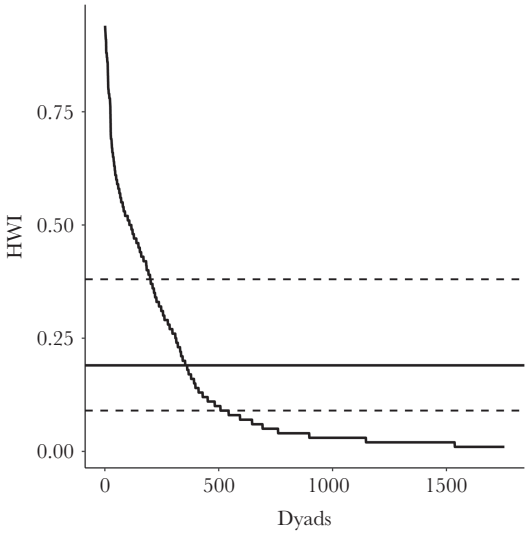

Figure 1

Change point analysis showing HWI values defining various levels of association. Distribution of adulthood HWI among 1,755 dyads of focal males and all their male associates, with lines representing change points. The continuous line denotes the HWI value of 0.19 separating males with social bonds above the line from males that associate occasionally below. The dashed line (HWI = 0.38) differentiates second-order from first-order alliance partners. The dotted line (HWI = 0.09) separates random associations from occasional associates.

Furthermore, the identified value of HWI ≥ 0.19 is well above average rates of association (mean HWI = 0.12 ± 0.17, excluding zeros, N = 1,755). Since HWIs were calculated per age period, they reflect increased levels of association over a prolonged period of time. Thus, this value indicates differentiated relationships among male dolphins that fit our definition of social bonds.

Male–male associations during adolescence and adulthood

During adolescence, pairwise relatedness significantly correlated with HWI (z = 2.974, P = 0.003; Table 2), indicating that adolescent focal males associated more frequently with related males. Age difference between a focal adolescent male and its close associates, as well as shared mtDNA haplotype, was not a significant predictor of association patterns during adolescence.

Once focal males reached adulthood, the averaged model indicated age similarity (z = 2.314, P = 0.021; Table 2) and adolescence HWI (z = 11.844, P < 0.001; Table 2) as significant predictors explaining patterns of association. These results suggested that adult males affiliated more often with males of similar age, as well as males with whom they already closely associated during adolescence (Figure 2). In contrast to the best adolescence models, pairwise relatedness was not included in the best adulthood models and, thus, did not hold explanatory power for male associations. As in the adolescence model, shared mtDNA haplotype was not significant.

Within-dyad relationship development

We identified 209 dyads (53%) that were close associates during adolescence and became alliance partners in adulthood (ASA). In contrast, relatively few dyads (N = 32, 8%) were close associates during adolescence without becoming alliance partners in adulthood (ASO). Notably, this was not due to some individuals missing from the adulthood dataset because of permanent emigration or death; instead, this result may reflect selective termination of social bonds. We found 152 dyads (39%) that became alliance partners during adulthood without having closely associated during adolescence (AO). This result implies a significant overall net gain of social bonds during the transition from adolescence to adulthood (mean close associates ± se adolescent males = 4.1 ± 3.2; mean alliance partners adulthood ± se = 6.1 ± 3.8; Poisson Generalized Linear Mixed Model, z = 4.414, se = 0.083, P < 0.001; Figure 3; Supplementary Table S1: Model 3). To ensure that this result was not biased by the conservative cutoff of HWI ≥ 0.19 for adolescence, we re-ran the change point analysis including adolescence HWIs > 0 only. The change point separating close associates during adolescence was found at HWI ≥ 0.15. On average, individual males still had significantly more social bonds in their adulthood (mean close associates ± se adolescence males = 4.9 ± 3.4; mean alliance partners ± se adulthood = 6.1 ± 3.8; Poisson Generalized Linear Mixed Model, z = 2.728, se = 0.078, P = 0.006).

The within-dyad relationship models (Models 4) showed no effect of either shared haplotype or pairwise relatedness on within-dyad relationship development between adolescence and adulthood (Table 3, *Hap.*, *r*). Hence, neither the persistence nor the formation of social bonds between adolescence and adulthood seemed to be influenced by relatedness. However, age difference was significant in 2 out of the 3 models (Table 3, Δage). Dyads that were close associates in adolescence but did not become alliance partners in adulthood (ASO) had a significantly larger age difference

Table 2

Results of averaged models on male association rates during adolescence and adulthood

	β		SE		*z*-value		*P*-value	
	Adolescent	Adult	Adolescent	Adult	Adolescent	Adult	Adolescent	Adult
Intercept	0.352	0.426	0.014	0.015	26.016	29.053	**< 0.001**	**< 0.001**
Relatedness	0.047	NA	0.016	NA	2.947	NA	**0.003**	NA
Haplotype	0.002	0.005	0.009	0.011	0.269	.474	0.788	0.636
$HWI_{Adolescent}$	NA	0.187	NA	0.016	NA	11.844	NA	**< 0.001**
Age difference	−0.019	−0.039	0.020	0.017	0.927	2.314	0.354	**0.0207**

Results of averaged Models 1a–c and averaged Models 2a–b (Table 1), investigating the effect of age similarity, HWI during the focal male's adolescence (Models 2), pairwise relatedness, and shared haplotypes on association patterns during adolescence and adulthood in males.
P-values in bold indicate significant results after Bonferroni correction.

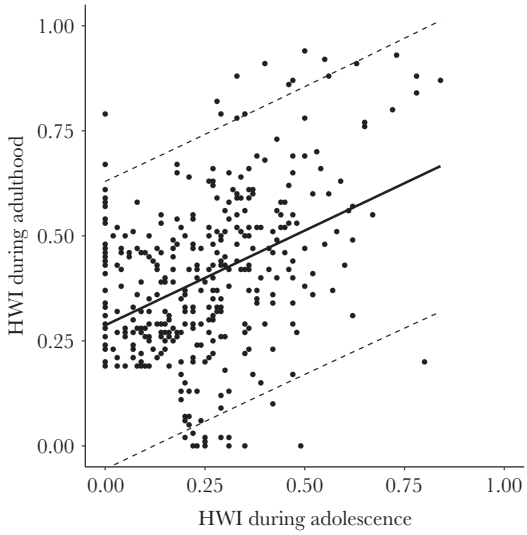

Figure 2
Positive correlation between adolescent and adult association rates within dyads. The association rate between an adolescent male and a second individual predicts the same dyad's association rate once a male matured into an adult, suggesting adolescent close associates foreshadow alliance partners (N = 361 dyads, Model 2). The dashed lines enclose the 95% confidence interval.

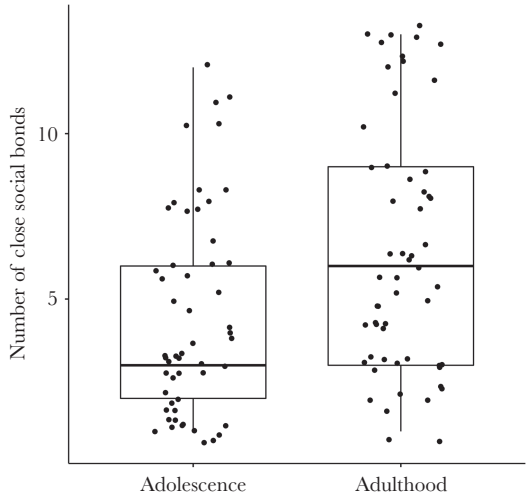

Figure 3
Increase in number of social bonds during the transition from adolescence to adulthood. Boxplot showing the average number of social bonds during the focal male's adolescence (i.e., number of close associates) and adulthood (i.e., number of alliance partners) including 95% confidence intervals. The average number of social bonds (HWI ≥ 0.19) is higher in a male's adult age bracket compared with its adolescence. This result suggests that males form additional social bonds during the transition into adulthood (Poisson Generalized Linear Mixed Model, including age period, i.e., adolescence or adulthood, as explanatory, number of close associates/alliance partners as dependent, and IDs as random variable, $P < 0.001$, $N = 118$, Model 3).

(mean ± sd = 8.6 ± 7.1 years) than dyads that were close associates during adolescence and became alliance partners later on (ASA, mean age difference 3.5 ± 3.4 years; Binomial Generalized Linear Mixed Model, $P < 0.001$). These results indicated that similarly aged males were more likely to form persistent social bonds and, thus, become alliance partners. We also found significantly larger age differences between males who were close associates during adolescence without becoming alliance partners (ASO, Binomial Generalized Linear Mixed Model, $P < 0.001$) compared with dyads that were alliance partners but had not been close associates (AO, mean age difference 3.8 ± 3.1 years). It appeared that adult males formed social bonds with other males of more similar age than they did during adolescence.

DISCUSSION

Affiliation history and age similarity predicted social bonds and thus alliance partnerships among adult male bottlenose dolphins. Individual social bonds formed during adolescence, particularly those among similar-aged males, persisted into adulthood. Kinship explained social bonds of adolescents, most likely due to bisexual philopatry (Krützen et al. 2004b; Tsai and Mann 2013). However, kinship did not predict the persistence of social bonds into adulthood or the existence of social bonds among adult males. Social bonds were maintained during the transition from adolescence to adulthood, yet, relatedness no longer predicted patterns of associations among adult dolphins. This effect is likely due to males increasing their number of social bonds between adolescence and adulthood.

Our finding that association rates during adolescence were positively correlated with relatedness may be best explained by associations with their mothers' related associates. In Shark Bay, adult females maintain high association rates with female relatives (Frère et al. 2010). This is in line with recent work that social networks in a large range of species, including bottlenose dolphins (Ilany and

Table 3
Results from within-dyad relationship analyses

	β			SE			*z*-value			*P*-value		
	r	Δage	Hap.	r	Δage	Hap.	r	Δage	Hap.	r	Δage	Hap.
ASA-AO	1.54	−0.10	0.36	1.06	0.05	0.20	1.45	−2.04	1.86	0.15	0.04	0.06
ASA-ASO	−0.11	−5.45	−0.67	5.89	1.11	1.50	−0.02	−4.90	−0.45	0.99	**<0.001**	0.66
ASO-AO	−20.08	1.38	3.37	11.80	0.24	2.13	−1.70	5.70	1.59	0.089	**<0.001**	0.11

Results of Models 4a–c, top to bottom.
ASA = close associates during adolescence and alliance partners in adulthood, ASO = close associates during adolescence without becoming alliance partners in adulthood, and AO = alliance partners in adulthood but not close associates during adolescence. r = pairwise relatedness, Δage = age difference in years, Hap. = shared or nonshared haplotype.
P-values in bold indicate significant results after Bonferroni correction.

Akçay 2016), were shaped by social inheritance of maternal bonds (Goldenberg et al. 2016; Kerhoas et al. 2016).

Simple rules based on proximity and familiarity, without requiring sophisticated mechanisms to distinguish kin from nonkin, may operate where partner choice is kin biased. This is found in many primate species characterized by female philopatry (e.g., cercopithecine primates, Chapais 2002). Persistent social bonds during the transition from adolescence to adulthood suggest that male dolphins might apply the same rules. Yet, the outcome is very different when individuals are expected to have access to a limited number of close relatives and where kin discrimination may be limited (Lewis et al. 2013). Moreover, in order to maximize individual fitness, the identification of competent and compatible alliance partners may be more important than kin-biased partner choice. Traits that might be valued in alliance partners include resource holding potential (Parker 1974), cooperativeness (Heinsohn and Packer 1995), including the propensity to return received favors (Taborsky et al. 2016), competence (Chapais 2006), and other characteristics, such as behavioral homophily (e.g., in chimpanzees, Massen and Koski 2014) or dominance rank (e.g., hyenas *Crocuta crocuta*, Smith et al. 2007). The amount of time that individuals have been associated and their relationship quality may also be important factors influencing partner choice, as recently shown in female mangabeys (*Cercocebus atys*) and chimpanzees (Mielke et al. 2018), as well as ravens (*Corvus corax*, Asakawa-Haas et al. 2016). Furthermore, a modeling approach as well as experimental evidence from humans suggests that individuals are more likely to cooperate with previously helpful partners (Wang et al. 2012; Campennì and Schino 2014). Multiple positive experiences with the same partners can thus lead to persisting social bonds, although this may not always be the case (Schweinfurth and Taborsky 2018).

Adult male bottlenose dolphins in Shark Bay primarily cooperate to sequester estrus females within consortships, or in conflicts over access to females with other alliances; the latter entailing risk of injury (Connor et al. 2011; Connor and Krützen 2015; Hamilton et al. 2019). Hence, a male's reproductive success is not only determined by his own competence but also by that of his alliance partners, and how well these individuals can synchronize and coordinate their joint behaviors (Connor et al. 2006). Evidence that males selectively choose partners for activities whose payoff is affected by partner competency, independently of kinship, has also been described in chimpanzees. Males will participate in alliances, take part in risky boundary patrols, or share meat with the males that they either associate with most often and trust to take the same risk (Watts and Mitani 2001), or with males of similar age and rank, rather than kin (Mitani et al. 2000, 2002b).

Bisexual philopatry and a slow life history, including a long developmental period, provide male dolphins in Shark Bay with opportunities to assess each other's competencies and compatibilities, as well as to form social bonds with desirable future alliance partners. Indeed, similar to chimpanzees (Kawanaka 1989; Pusey 1990), rats (*Rattus norvegicus*, Auger and Olesen 2009), and elephants (*Loxodonta africana*, Evans and Harris 2008), juvenile male dolphins in Shark Bay invest more time into social activities compared with females (Krzyszczyk et al. 2017). Social activities often involve playherding, a behavior where adolescents practice consortship behavior (Connor et al. 2000a), which potentially serves to increase efficacy of future consortships through joint practice or to identify valuable alliance partners.

The persistence of social bonds during the transition from adolescence to adulthood implies that the actual formation of alliances is a protracted process, likely starting years before males become reproductively active. Even though social bonds among unrelated, similar-aged males could also arise by demographic constraints without invoking partner choice (Connor et al. 2000b), our finding of persistent social bonds and the fact that they are subject to directional changes suggest otherwise. During the transition from adolescence to adulthood, focal males increased the overall number of social bonds with new social bonds typically arising among male dolphins of similar age. The few social bonds that were discontinued were observed among males with larger age disparities. Hence, male dolphins showed a preference for a larger number of similar-aged alliance partners.

In bats, individuals can benefit from expanding their social networks by forming social bonds with nonkin (*Desmodus rotundus*, Carter et al. 2017). Individuals with larger networks consisting of kin and nonkin alike coped better with partner loss compared with individuals who had smaller networks consisting predominantly of kin. Considering the importance of alliance partners for male dolphins, expanding the social network with valuable nonkin individuals might lead to an increase in reproductive success and, thus, fitness. Under such a scenario, males might benefit from a larger number of alliance partners composed of kin and nonkin alike. Because of the different constraints on reproductive success between the sexes, males might prefer a large number of competent but unrelated partners. This is not necessarily the case for females. Therefore, social bond formation might underlie different criteria in the two sexes and might explain why males form social bonds

with nonkin, whereas the social partners of females usually consist of kin (Möller et al. 2006; Frère et al. 2010).

Male bottlenose dolphins might apply similar rules based on proximity, homophily, and familiarity for alliance formation as other species. Due to their social system, however, these do not necessarily lead to kin-biased cooperation. Thus, rather than kin selection, we propose that other evolutionary mechanisms, such as forms of intra-species mutualism (Connor 1986), reciprocity (reviewed in Trivers 1971; Taborsky et al. 2016), or a combination thereof, should be invoked when explaining the evolution of social bonds in male bottlenose dolphins (reviewed in Connor 2010). Our results reveal that long-term familiarity and age similarity, but not necessarily relatedness, influence the formation of multilevel alliances in male Indo-Pacific bottlenose dolphins. With this study, we add to the growing body of research highlighting the occurrence of enduring social bonds, or friendships, in animals, independent of relatedness.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at *Behavioral Ecology* online.

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REFERENCES

Asakawa-Haas K, Schiestl M, Bugnyar T, Massen JJ. 2016. Partner choice in raven (*Corvus corax*) cooperation. PLoS One. 11:e0156962.
Auger AP, Olesen KM. 2009. Brain sex differences and the organisation of juvenile social play behaviour. J Neuroendocrinol. 21:519–525.
Bacher K, Allen S, Lindholm AK, Bejder L, Krützen M. 2010. Genes or culture: are mitochondrial genes associated with tool use in bottlenose dolphins (*Tursiops sp.*)? Behav Genet. 40:706–714.
Barton K. 2018. Multi-model inference. Package 'MuMIn'. R package version 1.40.4.
Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw. 67:1–48.
Boehm C. 2009. Hierarchy in the forest: the evolution of egalitarian behavior. Cambridge (MA): Harvard University Press.
Braun A, Bugnyar T. 2012. Social bonds and rank acquisition in raven nonbreeder aggregations. Anim Behav. 84:1507–1515.
Burnham KP, Anderson DR. 2003. Model selection and multimodel inference: a practical information-theoretic approach. Berlin (Germany): Springer Science & Business Media.
Cairns SJ, Schwager SJ. 1987. A comparison of association indices. Anim Behav. 35:1454–1469.
Cameron EZ, Setsaas TH, Linklater WL. 2009. Social bonds between unrelated females increase reproductive success in feral horses. Proc Natl Acad Sci USA. 106:13850–13853.
Campennì M, Schino G. 2014. Partner choice promotes cooperation: the two faces of testing with agent-based models. J Theor Biol. 344:49–55.
Caro TM. 1990. Cheetah mothers bias parental investment in favour of cooperating sons. Ethol Ecol Evol. 2:381–395.
Caro TM. 1994. Cheetahs of the Serengeti Plains: group living in an asocial species. Chicago (IL): University of Chicago Press.
Carter GG, Farine DR, Wilkinson GS. 2017. Social bet-hedging in vampire bats. Biology Letters. 13:20170112.
Chagnon NA. 1988. Life histories, blood revenge, and warfare in a tribal population. Science. 239:985–992.
Chapais B. 2002. The role of alliances in social inheritance of rank among female primates. In: Harcourt AH, de Waal FBM, editors. Coalitions and alliances in humans and other animals. Oxford: Oxford Science Publications. p. 29–59.
Chapais B. 2006. Kinship, competence and cooperation in primates. In: Kappeler PM, van Schaik CP, editors. Cooperation in primates and humans: mechanisms and evolution. Berlin (Heidelberg): Springer Berlin Heidelberg. p. 47–64.
Connor RC. 1986. Pseudo-reciprocity: investing in mutualism. Anim Behav. 34:1562–1566.
Connor RC. 2010. Cooperation beyond the dyad: on simple models and a complex society. Philos Trans R Soc Lond B Biol Sci. 365:2687–2697.
Connor RC, Cioffi WR, Randić S, Allen SJ, Watson-Capps J, Krützen M. 2017. Male alliance behaviour and mating access varies with habitat in a dolphin social network. Sci Rep. 7:46354.
Connor RC, Heithaus MR, Barre LM. 2001. Complex social structure, alliance stability and mating access in a bottlenose dolphin 'super-alliance'. Proc Biol Sci. 268:263–267.
Connor RC, Krützen M. 2015. Male dolphin alliances in Shark Bay: changing perspectives in a 30-year study. Anim Behav. 103:223–235.
Connor RC, Mann J, Read A, Wells RJ. 2000a. The bottlenose dolphin: social relationships in a fission-fusion society. In: Mann J, Connor RC, Tyack P, Whitehead H, editors. Cetacean societies: field studies of whales and dolphins. Chicago (IL): University of Chicago Press. p. 91–126.
Connor RC, Read AJ, Wrangham R. 2000b. Male reproductive strategies and social bonds. In: Mann J, Connor RC, Tyack P, Whitehead H, editors. Cetacean societies: field studies of dolphins and whales. Chicago (IL): University of Chicago Press. p. 247–269.
Connor RC, Smolker R, Bejder L. 2006. Synchrony, social behaviour and alliance affiliation in Indian Ocean bottlenose dolphins, *Tursiops aduncus*. Anim Behav. 72:1371–1378.
Connor RC, Smolker RA, Richards AF. 1992. Two levels of alliance formation among male bottlenose dolphins (*Tursiops* sp.). Proc Natl Acad Sci USA. 89:987–990.
Connor RC, Watson-Capps JJ, Sherwin WB, Krützen M. 2011. A new level of complexity in the male alliance networks of Indian Ocean bottlenose dolphins (*Tursiops* sp.). Biol Lett. 7:623–626.
Csillery K, Johnson T, Beraldi D, Clutton-Brock T, Coltman D, Hansson B, Spong G, Pemberton JM. 2006. Performance of marker-based relatedness estimators in natural populations of outbred vertebrates. Genetics. 173:2091–2101.
Diaz-Aguirre F, Parra GJ, Passadore C, Möller L. 2018. Kinship influences social bonds among male southern Australian bottlenose dolphins (*Tursiops cf. australis*). Behav Ecol Sociobiol. 72:190.
Díaz-Muñoz SL, Du Val EH, Krakauer AH, Lacey EA. 2014. Cooperating to compete: altruism, sexual selection and causes of male reproductive cooperation. Anim Behav. 88:67–78.
Dunbar RI. 2010. The social role of touch in humans and primates: behavioural function and neurobiological mechanisms. Neurosci Biobehav Rev. 34:260–268.
Dunnett CW. 1955. A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc. 50:1096–1121.
Evans KE, Harris S. 2008. Adolescence in male African elephants, *Loxodonta africana*, and the importance of sociality. Anim Behav. 76:779–787.

Faaborg J, Parker P, De Lay L, De Vries T, Bednarz J, Paz SM, Naranjo J, Waite T. 1995. Confirmation of cooperative polyandry in the Galapagos hawk (*Buteo galapagoensis*). Behav Ecol Sociobiol. 36:83–90.
Frère CH, Krützen M, Mann J, Watson-Capps JJ, Tsai YJ, Patterson EM, Connor R, Bejder L, Sherwin WB. 2010. Home range overlap, matrilineal and biparental kinship drive female associations in bottlenose dolphins. Anim Behav. 80:481–486.
Gerber L, Connor RC, King SL, Allen SJ, Wittwer S, Bizzozzero MR, Friedman WR, Kalberer S, Sherwin WB, Wild S, et al. 2019. Data from: affiliation history and age similarity predict alliance formation in adult male bottlenose dolphins. Dryad Digital Repository. doi: 10.5061/dryad.3n5tb2rcd.
Goldenberg SZ, Douglas-Hamilton I, Wittemyer G. 2016. Vertical transmission of social roles drives resilience to poaching in elephant networks. Curr Biol. 26:75–79.
Greenwood PJ. 1980. Mating systems, philopatry and dispersal in birds and mammals. Anim Behav. 28:1140–1162.
Grueber CE, Nakagawa S, Laws RJ, Jamieson IG. 2011. Multimodel inference in ecology and evolution: challenges and solutions. J Evol Biol. 24:699–711.
Grueter CC, Chapais B, Zinner D. 2012. Evolution of multilevel social systems in nonhuman primates and humans. Int J Primatol. 33:1002–1037.
Hamilton WD. 1964. The genetical evolution of social behaviour. I. J Theor Biol. 7:1–16.
Hamilton RA, Borcuch T, Allen SJ, Cioffi WR, Bucci V, Krützen M, Connor RC. 2019. Aggression varies with consortship rate and habitat in a dolphin social network. Behav Ecol Sociobiol. 73:141. doi:10.1007/s00265-019-2753-1.
Harcourt AH. 1992. Cooperation in conflicts - commonalities between humans and other animals. PLS. 11:251–259.
Heinsohn R, Packer C. 1995. Complex cooperative strategies in group-territorial African lions. Science. 269:1260–1262.
Henzi S, Lusseau D, Weingrill T, Van Schaik C, Barrett L. 2009. Cyclicity in the structure of female baboon social networks. Behav Ecol Sociobiol. 63:1015–1021.
Holt-Lunstad J, Smith TB, Layton JB. 2010. Social relationships and mortality risk: a meta-analytic review. PLoS Med. 7:e1000316.
Ilany A, Akçay E. 2016. Social inheritance can explain the structure of animal social networks. Nat Commun. 7:12084.
Kawanaka K. 1989. Age differences in social interactions of young males in a chimpanzee unit-group at the Mahale Mountains National Park, Tanzania. Primates. 30:285–305.
Kemper CM, Trentin E, Tomo I. 2014. Sexual maturity in male Indo-Pacific bottlenose dolphins (*Tursiops aduncus*): evidence for regressed/pathological adults. J Mamm. 95:357–368.
Kerhoas D, Kulik L, Perwitasari-Farajallah D, Agil M, Engelhardt A, Widdig A. 2016. Mother-male bond, but not paternity, influences male-infant affiliation in wild crested macaques. Behav Ecol Sociobiol. 70:1117–1130.
Killick R, Eckley IA. 2014. changepoint: an R package for changepoint analysis. J Stat Softw. 58:1–19.
King SL, Friedman WR, Allen SJ, Gerber L, Jensen FH, Wittwer S, Connor RC, Krützen M. 2018. Bottlenose dolphins retain individual vocal labels in multi-level alliances. Curr Biol. 28:1993–1999.e3.
Kokko H, Johnstone RA. 1999. Social queuing in animal societies: a dynamic model of reproductive skew. Proc R S B Biol Sci. 266:571–578.
Kopps AM, Ackermann CY, Sherwin WB, Allen SJ, Bejder L, Krützen M. 2014. Cultural transmission of tool use combined with habitat specializations leads to fine-scale genetic structure in bottlenose dolphins. Proc Biol Sci. 281:20133245.
Krützen M, Barré LM, Connor RC, Mann J, Sherwin WB. 2004a. 'O father: where art thou?'— Paternity assessment in an open fission–fusion society of wild bottlenose dolphins (*Tursiops sp.*) in Shark Bay, Western Australia. Mol Ecol. 13:1975–1990.
Krützen M, Barré LM, Möller LM, Heithaus MR, Simms C, Sherwin WB. 2002. A biopsy system for small cetaceans: darting success and wound healing in *Tursiops spp*. Mar Mamm Sci. 18:863–878.
Krützen M, Sherwin WB, Berggren P, Gales N. 2004b. Population structure in an inshore cetacean revealed by microsatellite and mtDNA analysis: bottlenose dolphins (*Tursiops sp.*) in Shark Bay, Western Australia. Mar Mamm Sci. 20:28–47.
Krützen M, Sherwin WB, Connor RC, Barré LM, Van de Casteele T, Mann J, Brooks R. 2003. Contrasting relatedness patterns in bottlenose dolphins (*Tursiops sp.*) with different alliance strategies. Proc Biol Sci. 270:497–502.
Krzyszczyk E, Mann J. 2012. Why become speckled? Ontogeny and function of speckling in Shark Bay bottlenose dolphins (*Tursiops sp.*). Mar Mamm Sci. 28:295–307.
Krzyszczyk E, Patterson EM, Stanton MA, Mann J. 2017. The transition to independence: sex differences in social and behavioural development of wild bottlenose dolphins. Anim Behav. 129:43–59.
Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. Package 'lmerTest'. R package version 2. J. Stat. Softw. 82:13.
Langergraber KE, Mitani JC, Vigilant L. 2007. The limited impact of kinship on cooperation in wild chimpanzees. Proc Natl Acad Sci USA. 104:7786–7790.
Langergraber KE, Mitani JC, Watts DP, Vigilant L. 2013. Male–female socio-spatial relationships and reproduction in wild chimpanzees. Behav Ecol Sociobiol. 67:861–873.
Lewis JS, Wartzok D, Heithaus M, Krützen M. 2013. Could relatedness help explain why individuals lead in bottlenose dolphin groups? PLoS One. 8:e58162.
Mann J, Connor RC, Barre LM, Heithaus MR. 2000a. Female reproductive success in bottlenose dolphins (*Tursiops sp.*): life history, habitat, provisioning, and group-size effects. Behav Ecol. 11:210–219.
Mann J, Connor RC, Tyack P, Whitehead H. 2000b. Cetacean societies: field studies of dolphins and whales. Chicago (IL): University of Chicago Press.
Massen JJM. 2017. Friendships in animals. In: Vonk J, Shackelford T, editors. Encyclopedia of animal cognition and behavior. Cham (Switzerland): Springer International Publishing. p. 1–6.
Massen JJM, Koski SE. 2014. Chimps of a feather sit together: chimpanzee friendships are based on homophily in personality. Evol Hum Behav. 35:1–8.
McDonald DB, Potts WK. 1994. Cooperative display and relatedness among males in a lek-mating bird. Science. 266:1030–1032.
Mielke A, Preis A, Samuni L, Gogarten JF, Wittig RM, Crockford C. 2018. Flexible decision-making in grooming partner choice in sooty mangabeys and chimpanzees. R Soc Open Sci. 5:172143.
Mitani JC, Merriwether DA, Zhang C. 2000. Male affiliation, cooperation and kinship in wild chimpanzees. Anim Behav. 59:885–893.
Mitani JC. 2009. Male chimpanzees form enduring and equitable social bonds. Anim Behav. 77:633–640.
Mitani JC, Watts DP, Muller MN. 2002a. Recent developments in the study of wild chimpanzee behavior. Evol Anthropol. 11:9–25.
Mitani JC, Watts DP, Pepper JW, Merriwether DA. 2002b. Demographic and social constraints on male chimpanzee behaviour. Anim Behav. 64:727–737.
Möller LM, Beheregaray LB, Allen SJ, Harcourt RG. 2006. Association patterns and kinship in female Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) of southeastern Australia. Behav Ecol Sociobiol. 61:109–117.
Möller LM, Beheregaray LB, Harcourt RG, Krützen M. 2001. Alliance membership and kinship in wild male bottlenose dolphins (*Tursiops aduncus*) of southeastern Australia. Proc Biol Sci. 268:1941–1947.
Noë R. 1992. Alliance formation among male baboons: shopping for profitable partners. In: Harcourt AH, De Waal FBM, editors. Coalitions and alliances in humans and other animals. Oxford: Oxford University Press.
Ostner J, Schülke O. 2014. The evolution of social bonds in primate males. Behaviour. 151:871.
Owen ECG, Wells RS, Hofmann S. 2002. Ranging and association patterns of paired and unpaired adult male Atlantic bottlenose dolphins, *Tursiops truncatus*, in Sarasota, Florida, provide no evidence for alternative male strategies. Can J Zool. 80:2072–2089.
Packer C, Gilbert DA, Pusey AE, O'Brieni SJ. 1991. A molecular genetic analysis of kinship and cooperation in African lions. Nature. 351:562–565.
Parker GA. 1974. Assessment strategy and the evolution of fighting behaviour. J Theor Biol. 47:223–243.
Parsons KM, Durban JW, Claridge DE, Balcomb KC, Noble LR, Thompson PM. 2003. Kinship as a basis for alliance formation between male bottlenose dolphins, *Tursiops truncatus*, in the Bahamas. Anim Behav. 66:185–194.
Patzelt A, Kopp GH, Ndao I, Kalbitzer U, Zinner D, Fischer J. 2014. Male tolerance and male-male bonds in a multilevel primate society. Proc Natl Acad Sci USA. 111:14740–14745.

Pope TR. 1990. The reproductive consequences of male cooperation in the red howler monkey: paternity exclusion in multi-male and single-male troops using genetic markers. Behav Ecol Sociobiol. 27:439–446.
Poston JP. 1997. Dominance, access to colonies, and queues for mating opportunities by male boat-tailed grackles. Behav Ecol Sociobiol. 41:89–98.
Pusey AE. 1990. Behavioural changes at adolescence in chimpanzees. Behaviour. 115:203–246.
Qi X-G, Huang K, Fang G, Grueter CC, Dunn DW, Li Y-L, Ji W, Wang X-Y, Wang R-T, Garber PA, et al. 2017. Male cooperation for breeding opportunities contributes to the evolution of multilevel societies. Proc R Soc B Biol Sci. 284:20171480.
Randić S, Connor RC, Sherwin WB, Krützen M. 2012. A novel mammalian social structure in Indo-Pacific bottlenose dolphins (*Tursiops sp.*): complex male alliances in an open social network. Proc Biol Sci. 279:3083–3090.
R Core Team. 2017. R: a language and environment for statistical computing. Version 3.4.0. Vienna (Austria): R Foundation for Statistical Computing.
Schino G, Aureli F. 2017. Reciprocity in group-living animals: partner control versus partner choice. Biol Rev Camb Philos Soc. 92:665–672.
Schülke O, Bhagavatula J, Vigilant L, Ostner J. 2010. Social bonds enhance reproductive success in male macaques. Curr Biol. 20:2207–2210.
Schweinfurth MK, Neuenschwander J, Engqvist L, Schneeberger K, Rentsch AK, Gygax M, Taborsky M. 2017. Do female Norway rats form social bonds? Behav Ecol Sociobiol. 71:98.
Schweinfurth MK, Taborsky M. 2018. Relatedness decreases and reciprocity increases cooperation in Norway rats. Proc R Soc B Biol Sci. 285:20180035.
Silk JB, Alberts SC, Altmann J. 2003. Social bonds of female baboons enhance infant survival. Science. 302:1231–1234.
Silk JB. 2002. Using the'F'-word in primatology. Behaviour. 139:421.
Silk JB, Beehner JC, Bergman TJ, Crockford C, Engh AL, Moscovice LR, Wittig RM, Seyfarth RM, Cheney DL. 2009. The benefits of social capital: close social bonds among female baboons enhance offspring survival. Proc Biol Sci. 276:3099–3104.
Silk JB, Beehner JC, Bergman TJ, Crockford C, Engh AL, Moscovice LR, Wittig RM, Seyfarth RM, Cheney DL. 2010. Strong and consistent social bonds enhance the longevity of female baboons. Curr Biol. 20:1359–1361.
Smith JE. 2014. Hamilton's legacy: kinship, cooperation and social tolerance in mammalian groups. Anim Behav. 92:291–304.
Smith JE, Memenis SK, Holekamp KE. 2007. Rank-related partner choice in the fission–fusion society of the spotted hyena (*Crocuta crocuta*). Behav Ecol Sociobiol. 61:753–765.
Smolker RA, Richards AF, Connor RC, Pepper JW. 1992. Sex differences in patterns of association among Indian Ocean bottlenose dolphins. Behaviour. 123:38–69.
Smuts B, Mann J. 1999. Behavioral development in wild bottlenose dolphin newborns (*Tursiops* sp.). Behaviour. 136:529–566.
Stanton MA, Gibson QA, Mann J. 2011. When mum's away: a study of mother and calf ego networks during separations in wild bottlenose dolphins (*Tursiops* sp.). Animal Behaviour. 82:405–412.
Taborsky M. 1994. Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. Adv Study Behav. 23:1–100.
Taborsky M, Frommen JG, Riehl C. 2016. Correlated pay-offs are key to cooperation. Philos Trans R Soc Lond B Biol Sci. 371:20150084.
Thompson NA. 2019. Understanding the links between social ties and fitness over the life cycle in primates. Behaviour. 156:859.
Trivers R. 1971. The evolution of reciprocal altruism. Q Rev Biol. 46:35–57.
Trivers R. 1972. Parental investment and sexual selection. In: Campbell B, editor. Sexual selection and the descent of man 1871 – 1971. Chicago (IL): Aldine. p. 136–179.
Tsai YJJ, Mann J. 2013. Dispersal, philopatry, and the role of fission-fusion dynamics in bottlenose dolphins. Mar Mamm Sci. 29:261–279.
Wang J. 2007. Triadic IBD coefficients and applications to estimating pairwise relatedness. Genet Res. 89:135–153.
Wang J. 2011. Coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol Ecol Resour. 11:141–145.
Wang J, Suri S, Watts DJ. 2012. Cooperation and assortativity with dynamic partner updating. Proc Natl Acad Sci USA. 109:14363–14368.
Watts DP. 1998. Coalitionary mate guarding by male chimpanzees at Ngogo, Kibale National Park, Uganda. Behav Ecol Sociobiol. 44:43–55.
Watts DP, Mitani JC. 2001. Boundary patrols and intergroup encounters in wild chimpanzees. Behaviour. 138:299–327.
Weidt A, Hofmann SE, König B. 2008. Not only mate choice matters: fitness consequences of social partner choice in female house mice. Anim Behav. 75:801–808.
Whitehead H. 2008. Analyzing animal societies. Chicago (IL): University Of Chicago Press.
Whitehead H. 2009. SOCPROG programs: analysing animal social structures. Behav Ecol Sociobiol. 63:765–778.
Wiszniewski J, Brown C, Möller LM. 2012a. Complex patterns of male alliance formation in a dolphin social network. J Mamm. 93:239–250.
Wiszniewski J, Corrigan S, Beheregaray LB, Möller LM. 2012b. Male reproductive success increases with alliance size in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*). J Anim Ecol. 81:423–431.
Wittemyer G, Douglas-Hamilton I, Getz WM. 2005. The socioecology of elephants: analysis of the processes creating multitiered social structures. Anim Behav. 69:1357–1371.
Zuur AF, Ieno EN, Smith GM. 2007. Analyzing ecological data. New York: Springer Science & Business Media.
Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009. Mixed effects models and extensions in ecology with R. New York: Springer.

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Animal behaviour

Multi-network-based diffusion analysis reveals vertical cultural transmission of sponge tool use within dolphin matrilines

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Behavioural differences among social groups can arise from differing ecological conditions, genetic predispositions and/or social learning. In the past, social learning has typically been inferred as responsible for the spread of behaviour by the exclusion of ecological and genetic factors. This 'method of exclusion' was used to infer that 'sponging', a foraging behaviour involving tool use in the bottlenose dolphin (*Tursiops aduncus*) population in Shark Bay, Western Australia, was socially transmitted. However, previous studies were limited in that they never fully accounted for alternative factors, and that social learning, ecology and genetics are not mutually exclusive in causing behavioural variation. Here, we quantified the importance of social learning on the diffusion of sponging, for the first time explicitly accounting for ecological and genetic factors, using a multi-network version of 'network-based diffusion analysis'. Our results provide compelling support for previous findings that sponging is vertically socially transmitted from mother to (primarily female) offspring. This research illustrates the utility of social network analysis in elucidating the explanatory mechanisms behind the transmission of behaviour in wild animal populations.

1. Introduction

Various mechanisms can be responsible for causing behavioural differences among social groups or populations [1]. The *cultural hypothesis* states that behavioural variation is the result of social transmission of different behavioural innovations. The *ecological hypothesis*, on the other hand, proposes that behavioural differences among groups can be attributed to differing ecological conditions. Finally, the *genetic hypothesis* assumes that different groups are genetically predisposed to behave in different ways [1].

The last few decades have seen increasing interest in animal cultural phenomena, i.e. behaviours that are socially transmitted among conspecifics [1]. Various methods have been used to identify social learning in animal populations. For example, the *method of exclusion* (also termed *group contrast method*, or *ethnographic method*)—commonly used among primatologists in the past, e.g. [2,3]—identifies patterns of variation in the behavioural repertoire of the population in question and infers social transmission as at least partly responsible for differing behaviours by excluding genetic and ecological differences as sufficient explanations [4, p. 132].

The method of exclusion has also been used to assess patterns of transmission of 'sponging', a foraging behaviour involving tool use in a population of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in Shark Bay, Western Australia [5]. This behaviour involves dolphins carrying conical sponges as protective 'gloves' on their rostra when foraging for buried prey [6]. Sponging is female-biased, and almost all sponging dolphins possess the same mitochondrial haplotype, i.e. belong to the same matriline [5,7]. As the deep-water channels where sponging occurs were used by both 'spongers' and 'non-spongers', a purely ecological explanation seemed unlikely [5]. By considering 10 different pathways of potential genetic inheritance (x-linked and autosomal), Krützen *et al*. [5] inferred that sponging was vertically socially transmitted from mother to female offspring.

The method of exclusion has been criticized, however, with considerable debate over its utility [8–10]. Laland & Janik [9] argued that it is impossible to take all plausible explanations for the spread of behaviour into account, and therefore, that social learning can never be inferred with absolute certainty, leading to increased rates of false claims of culture [4]. Furthermore, they argued that social learning, ecology and genetics are not necessarily mutually exclusive [9,10]. Instead, they can simultaneously shape behaviour in a population, warranting a more nuanced approach to disentangle the relative contributions of the three drivers of behavioural variation.

In an attempt to resolve the animal cultures debate, more quantitative methods to infer social learning have been developed. For example, using repertoire-based methods on long-term behavioural data from 11 orangutan (*Pongo* spp.) populations, Krützen *et al*. [11] showed that neither uniquely genetic nor ecological components explained the total observed variance with regard to putative cultural elements, corroborating a cultural explanation. Further, 'network-based diffusion analysis' (NBDA) [12,13], a network-based approach allowing the quantification of the importance of social learning on the spread of behaviour, has been used increasingly in recent years to detect and quantify social learning in animal populations, e.g. [14,15]. NBDA infers social transmission if the spread of a behaviour follows the social network, assuming that more closely associated individuals have more opportunities to learn from each other [13,16]. Multi-network NBDA allows the inclusion of several different networks to quantify the relative importance of transmission along different pathways [17].

Here, we used multi-network NBDA to quantify the relative importance of social learning, ecological factors and genetic relatedness on the spread of sponge tool use in the dolphin population of Shark Bay, Western Australia. Furthermore, we distinguished between different pathways of social learning, namely vertical (between mother and offspring) and horizontal/oblique learning (among peers/between older and younger generations, respectively).

2. Material and methods

(a) Field methods

We collected association and behavioural data during boat-based surveys using standardized sampling methods for cetaceans between 2007 and 2018 in the western gulf of Shark Bay, Western Australia. On approach to each dolphin group, we recorded GPS location, determined group composition during the first 5 min of each encounter using long-established photo-identification techniques [18] and recorded predominant group behaviour. All occurrences of sponging were recorded and an individual was deemed a 'sponger' once it had been seen carrying a sponge on at least two independent occasions. Biopsy samples were taken on an opportunistic basis using a system designed specifically for sampling cetaceans [19].

(b) Genetic methods

To test for a genetic predisposition for developing sponging behaviour, we obtained a measure of genetic biparental relatedness for each dyad. Individuals for which biopsies were available ($N = 295$) were genetically sexed [20] and genotypes determined based on 27 microsatellite markers (electronic supplementary material, table S1). Using COANCESTRY 1.0.1.7 [21], we calculated dyadic biparental relatedness based on genotypes for individuals with no more than three microsatellite loci missing ($N = 293$), using the estimator TrioML [22] (electronic supplementary material). With a cut-off point of seven sightings (see below), genetic data were available on 226 out of 415 individuals, resulting in 25 425 unique dyads. For the remaining 189 individuals where no genetic information was available (60 480 dyads), we used the population average relatedness of 0.043.

We also statistically controlled for a correlation between matriline membership and sponging behaviour by sequencing a 468 bp-long fragment of the mitochondrial DNA (mtDNA) control region (d-loop) to assign dolphins to mtDNA haplotypes [23].

(c) Network constructions and network-based diffusion analysis

To assess the relative importance of social learning, ecological factors and genetics in promoting the spread of sponging, we ran multi-network NBDA [17] using four different networks (NBDA package v. 0.6.1 [24] in R 3.5.1 [25]). The first social network assessed vertical learning between mother and offspring, with entries of 1 between mother and known offspring and all other connections set to 0. We created the network based on behaviourally and genetically identified mother–offspring pairs ($N = 294$; electronic supplementary material). The second social network allowed for horizontal/oblique (henceforth 'horizontal') learning using dyadic association strengths (simple ratio index [26]) among all individuals but excluding mother–offspring associations, which were set to 0. Sightings of the same or a subset of the original group within 2 h were excluded. Association matrices were created using R package 'asnipe' [27]. The third, ecological network contained dyadic home range overlap as a proxy of the environmental similarity experienced by individuals. We created home ranges using individual GPS locations based on 95% Epanechnikov kernel density estimates ('adehabitatHR' [28]) with a customized smoothing factor (electronic supplementary material). Dyadic home range overlap (95%) was calculated using the 'utilization distribution overlap index' (adehabitatHR) [28,29]. Finally, the fourth network contained measures of dyadic biparental genetic relatedness among individuals. Since NBDA infers social learning if a behaviour follows the social network, there is a trade-off between sample size and data quality. Dropping individuals with few sightings can increase certainty about the strengths of connections but, at the same time, decrease the power of NBDA to reliably detect social learning if linking individuals are removed [30]. We ran a simulation to select a threshold that maximizes the power of NBDA to detect social learning, revealing maximum power at seven sightings (electronic supplementary material) [30]. In all networks, we therefore only considered individuals with a minimum of seven observations.

We then applied the 'order of acquisition diffusion analysis' (OADA) variant of NBDA [13] (electronic supplementary

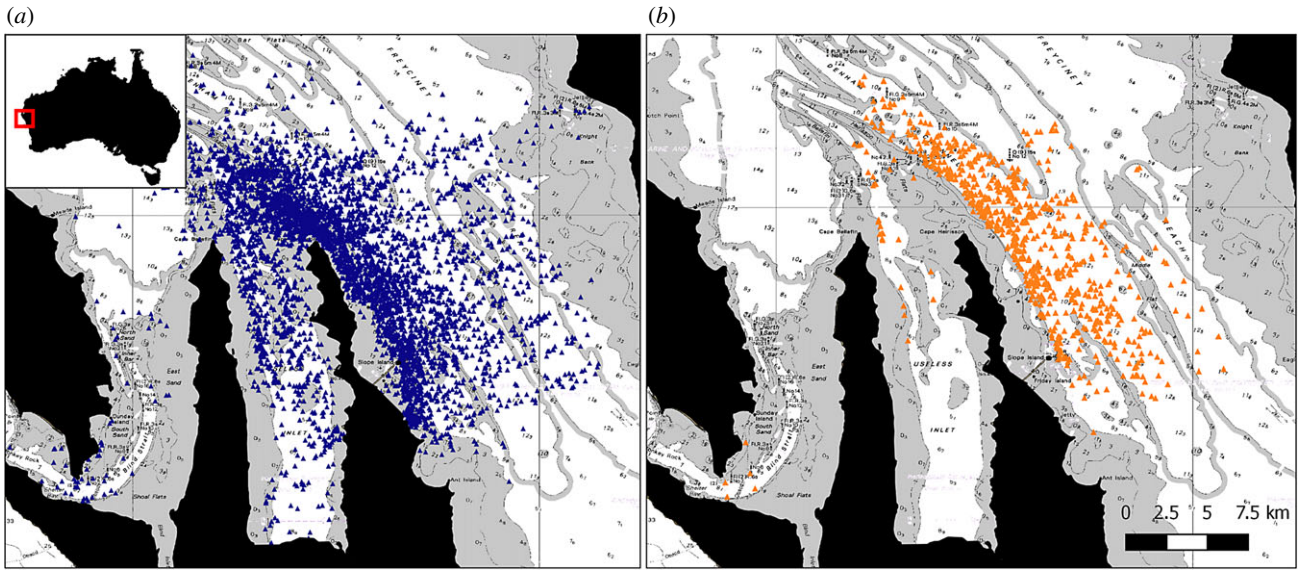

Figure 1. Locations of (*a*) all dolphin groups encountered in the western gulf of Shark Bay between 2007 and 2018; and (*b*) observations of sponging behaviour, which primarily occurred in deep (greater than 10 m) water channels (white areas).

material). For several individuals, the order of acquisition of sponging was unknown, as they were likely already spongers when first encountered. In NBDA models, such individuals can be taken to be 'informed' at the start of the diffusion (termed 'demonstrators') [13]. We considered all individuals as demonstrators who had been seen carrying a sponge within the first two encounters where predominant group behaviour was foraging. We argue that an individual's information state can be determined with reasonable certainty after two sightings, given spongers carry sponges 96% of the time when foraging [31]. Maternity data were unavailable for nine individuals who acquired sponging after 2007. These nine individuals were excluded as learners, but we allowed for other individuals having learned from these spongers (electronic supplementary material).

We included several individual-level variables (ILVs) with potential influence on the learning rate: sex; average water depth of each individual's sightings (a proxy for habitat use, since sponging occurs in deep-water channels [32]); average group size (since sponging is a solitary activity [31]) and mitochondrial haplotype as a reduced two-level factor (either haplotype E (=sponging haplotype in the western gulf [7]), or other) to avoid overfitting of models. Sex was determined genetically and/or by the presence of a dependent calf for females. In an NBDA, the strength of transmission through a network (s parameter) is estimated relative to a baseline rate of asocial learning. This baseline was set to the mean of all continuous variables, at the mid-point between males and females, and haplotype E (set as the reference level for this factor).

We fitted OADA with and without transmission through the networks and with all possible combinations of networks and ILVs [13]. Thereby, ILVs were allowed to influence both social and asocial learning rates independently ('unconstrained' models [4]; electronic supplementary material). Support for each model was calculated based on the Akaike information criterion corrected for sample size (AICc) [33]. To provide a more robust inference about strength of transmission for the different networks and the influence of ILVs, model averaging methods were employed [33]. We calculated 95% confidence intervals (CI) for model parameters using the profile likelihood method, conditional on the best performing model (electronic supplementary material).

3. Results

Between 2007 and 2018, 5300 dolphin groups were encountered in the western gulf of Shark Bay and more than 1000 different dolphins identified (figure 1*a*). Sponging was observed on 825 occasions and restricted to the deep-water channels within the study area (figure 1*b*). A total of 76 individuals were identified as spongers, of which 49 were confirmed female, 14 male and 13 of unknown sex.

After removal of individuals with fewer than seven sightings, as well as eight offspring that were either dependent calves at the time of analyses or had died before weaning, 415 individuals remained, of which 62 were spongers (18 learners, of which nine were removed due to missing maternity data, and 44 demonstrators). All spongers with maternity data available were born to sponging mothers. All spongers with genetic data available carried haplotype E, with one exception: a male sponger with haplotype H (but see electronic supplementary material).

Multi-network NBDA revealed most support for models with transmission through the vertical social network ($\sum w_i = 0.837$), while asocial learning, and transmission through the horizontal, ecological or genetic network (or any combination of the four networks) received much less support ($\sum w_i < 0.1$; figure 2). In the best performing model, which included vertical social transmission and sex influencing social learning, s (the rate of social transmission from mothers to offspring) was estimated to be 1.23×10^{10} times greater than the rate of asocial learning (95% CI [33.1; infinity]; $\sum w_i = 0.425$). The social learning rate was an estimated 126 times higher for females than males (95% CI [9.5; 2897]; $\sum w_i = 0.975$). This corresponds to approximately 100% of spongers learning sponging socially from their mothers (95% CI [98.9; 100]). The average group size, average water depth and haplotype did not influence social or asocial learning rate (all $\sum w_i < 0.5$; electronic supplementary material).

4. Discussion

We applied multi-network NBDA to sponging behaviour, revealing overwhelming support for social transmission through the vertical mother–offspring network, with little or no support for transmission through the horizontal association, ecological or genetic networks. Moreover, despite the restriction of sponging to channel habitat [32,34], our analysis

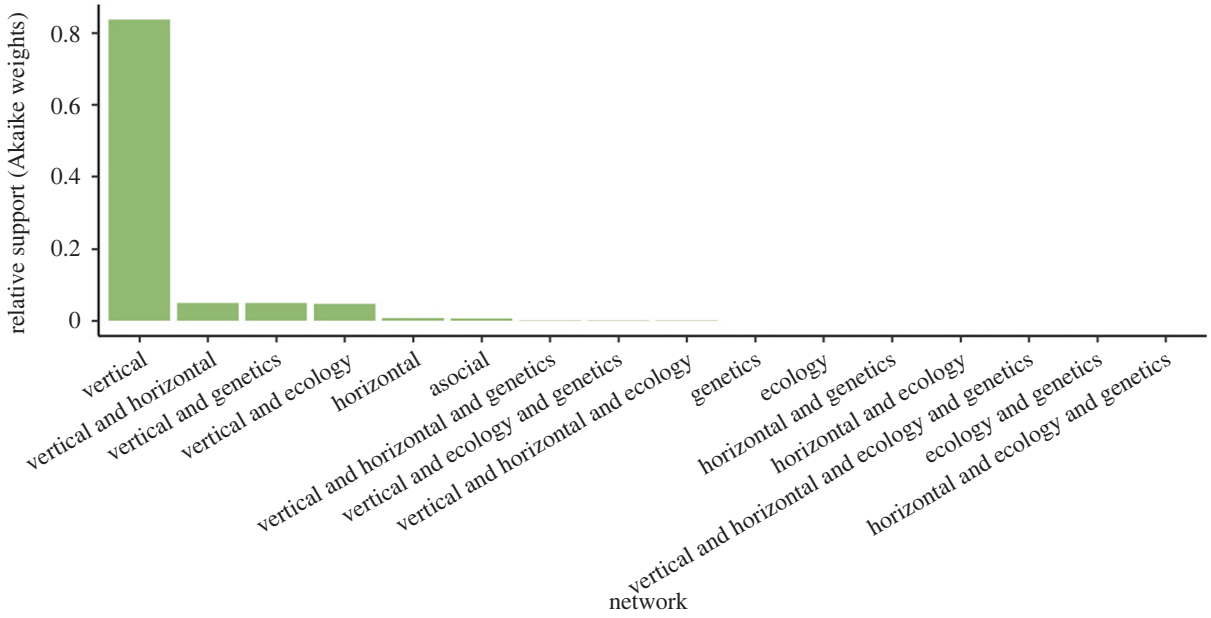

Figure 2. Multi-network NBDA revealed most support (based on Akaike weights) for transmission of sponging through the vertical social network, while transmission through the horizontal, ecological and relatedness networks (or combinations thereof) received little support.

suggests that ecological factors play only a minor role once vertical social learning has been taken into account.

Low support for transmission through the genetic network confirms previous findings that sponging individuals in the western gulf are not more closely related than expected by chance [7]. This stands in contrast with findings from the eastern gulf of Shark Bay, where spongers show higher relatedness than the population average, suggesting a more recent common ancestry [5].

We further confirm a previously documented female sex-bias [7,31,35], which is presumably due to differing sex-specific reproductive strategies between males and females [31]. After weaning, male dolphins must focus on forming multi-male alliances to coerce and consort oestrous females [36–38]. This requires significant investment in social relationships and is, therefore, largely incompatible with a time-consuming, solitary and difficult-to-master activity like sponging [31,39]. Meanwhile, female offspring are expected to invest more into developing foraging skills to maximize food intake compared to male offspring [40,41]. Alternatively, Zefferman [42] proposed that the female sex-bias could be the result of a maternal teaching strategy, arguing that teaching a daughter would result in higher long-term fitness for a female: a potential advantage of sponging for a son would last only one generation, while a daughter can pass on the behaviour to subsequent generations which all gain potential benefits associated with sponging. Just 22% of spongers with known sex in the western gulf were males, which corresponded to previously suggested proportions of male offspring learning sponging from their mothers in Shark Bay's eastern gulf ([31], but see [43]).

Given haplotype similarity among spongers, some researchers have argued that mitochondrial genes themselves might predispose dolphins to learning the sponging behaviour [9]. However, we find no evidence that being a member of a particular mtDNA matriline has an effect on the rate at which dolphins learn sponging, as per previous research [44]. Our findings instead support the hypothesis that maternal vertical transmission of both the sponging behaviour and mtDNA results in haplotype similarity among spongers, a phenomenon referred to as 'cultural hitchhiking'—a form of gene–culture coevolution in which a neutral genetic locus is inherited in parallel with a matrilineally transmitted cultural behaviour [45].

McElreath & Strimling's [46] mathematical models predict the conditions for the evolution of purely vertical transmission, concluding that 'neither [vertical nor oblique] transmission should be expected to dominate the other across all domains'. Sponging is just one foraging strategy exhibited by the dolphins, and other strategies may be transmitted obliquely and horizontally. Following McElreath & Strimling's models [46], we suggest that sponging is transmitted vertically because either (i) the relevant environment (e.g. availability of sponges) may be stable, or (ii) it may only be possible for a dolphin to learn sponging from its mother, if, for example, it requires repeated observations from close quarters.

The application of multi-network NBDA to sponging behaviour in the dolphins of western Shark Bay allowed us to quantify the effects of social learning on behaviour, while explicitly accounting for the influence of ecological and genetic factors for the first time. Documenting a strong effect of vertical social learning from mother to offspring, our findings provide compelling quantitative evidence to support the claim that sponging is a case of vertically transmitted culture in the bottlenose dolphins of Shark Bay [5].

Ethics. Permits for the use of animals for scientific purposes were granted by the Department of Biodiversity, Conservation and Attractions (SF002958; SF010888; SF10388; SF002958; SF010774; 08-000920-1; 08-000706-3) and the Department of Agriculture and Food (U 10/2015-2018). The animal ethics committees of the University of Western Australia, Murdoch University and the University of Zurich provided approvals for the ethical treatment of animals in scientific research (R2649/14; RA/3/100/1449; RA/3/100/1464).

Data accessibility. Code is available in electronic supplementary material, and data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.sc26m6c [47].

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References

1. Boyd R, Richerson PJ. 1995 Why culture is common, but cultural evolution is rare. *Proc. Br. Acad.* **88**, 77–93. (doi:citeulike-article-id:1339814)
2. van Schaik CP, Ancrenaz M, Borgen G, Galdikas B, Knott CD, Singleton I, Suzuki A, Utami SS, Merrill M. 2003 Orangutan cultures and the evolution of material culture. *Science* **299**, 102–105. (doi:10.1126/science.1078004)
3. Whiten A, Goodall J, McGrew WC, Nishida T, Reynolds V, Sugiyama Y, Tutin CE, Wrangham RW, Boesch C. 1999 Cultures in chimpanzees. *Nature* **399**, 682–685. (doi:10.1038/21415)
4. Hoppitt WJE, Laland KN. 2013 *Social learning: an introduction to mechanisms, methods, and models*. Princeton, NJ: Princeton University Press.
5. Krützen M, Mann J, Heithaus MR, Connor RC, Bejder L, Sherwin WB. 2005 Cultural transmission of tool use in bottlenose dolphins. *Proc. Natl Acad. Sci. USA* **102**, 8939–8943. (doi:10.1073/pnas.0500232102)
6. Smolker R, Richards A, Connor R, Mann J, Berggren P. 1997 Sponge carrying by dolphins (Delphinidae, *Tursiops* sp.): a foraging specialization involving tool use? *Ethology* **103**, 454–465. (doi:10.1111/j.1439-0310.1997.tb00160.x)
7. Kopps AM, Krützen M, Allen SJ, Bacher K, Sherwin WB. 2014 Characterizing the socially transmitted foraging tactic 'sponging' by bottlenose dolphins (*Tursiops* sp.) in the western gulf of Shark Bay, Western Australia. *Mar. Mammal Sci.* **30**, 847–863. (doi:10.1111/mms.12089)
8. Krützen M, van Schaik C, Whiten A. 2007 The animal cultures debate: response to Laland and Janik. *Trends Ecol. Evol.* **22**, 6. (doi:10.1016/j.tree.2006.10.011)
9. Laland KN, Janik VM. 2006 The animal cultures debate. *Trends Ecol. Evol.* **21**, 542–547. (doi:10.1016/j.tree.2006.06.005)
10. Laland K, Janik VM. 2007 Response to Krützen *et al*.: Further problems with the 'method of exclusion'. *Trends Ecol. Evol.* **22**, 7. (doi:10.1016/j.tree.2006.10.010)
11. Krützen M, Willems EP, van Schaik CP. 2011 Culture and geographic variation in orangutan behavior. *Curr. Biol.* **21**, 1808–1812. (doi:10.1016/j.cub.2011.09.017)
12. Franz M, Nunn CL. 2009 Network-based diffusion analysis: a new method for detecting social learning. *Proc. R. Soc. B* **276**, 1829–1836. (doi:10.1098/rspb.2008.1824)
13. Hoppitt W, Boogert NJ, Laland KN. 2010 Detecting social transmission in networks. *J. Theor. Biol.* **263**, 544–555. (doi:10.1016/j.jtbi.2010.01.004)
14. Allen J, Weinrich M, Hoppitt W, Rendell L. 2013 Network-based diffusion analysis reveals cultural transmission of lobtail feeding in humpback whales. *Science* **340**, 485–488. (doi:10.1126/science.1231976)
15. Hobaiter C, Poisot T, Zuberbühler K, Hoppitt W, Gruber T. 2014 Social network analysis shows direct evidence for social transmission of tool use in wild chimpanzees. *PLoS Biol.* **12**, e1001960. (doi:10.1371/journal.pbio.1001960)
16. Coussi-Korbel S, Fragaszy D. 1995 On the relation between social dynamics and social learning. *Anim. Behav.* **50**, 1441–1453. (doi:10.1016/0003-3472(95)80001-8)
17. Farine DR, Aplin LM, Sheldon BC, Hoppitt W. 2015 Interspecific social networks promote information transmission in wild songbirds. *Proc. R. Soc. B* **282**, 20142804. (doi:10.1098/rspb.2014.2804)
18. Würsig B, Jefferson T. 1990 Methods of photo-identification for small cetaceans. *Reports Int. Whal. Comm.* **12**, 43–52.
19. Krützen M, Barré L, Möller L, Heithaus M, Simms C, Sherwin W. 2002 A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Mar. Mammal Sci.* **18**, 863–878. (doi:10.1111/j.1748-7692.2002.tb01078.x)
20. Gilson A, Syvanen M, Levine K, Banks J. 1998 Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. *Calif. Fish Game* **84**, 159–169.
21. Wang J. 2011 COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Mol. Ecol. Resour.* **11**, 141–145. (doi:10.1111/j.1755-0998.2010.02885.x)
22. Wang J. 2007 Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genet. Res.* **89**, 135–153. (doi:10.1017/S0016672307008798)
23. Krützen M, Sherwin W, Berggren P, Gales N. 2004 Population structure in an inshore cetacean revealed by microsatellite and mtDNA analysis: bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. *Mar. Mammal Sci.* **20**, 28–47. (doi:10.1111/j.1748-7692.2004.tb01139.x)
24. Hoppitt WJE. 2018 NBDA: a package for implementing network-based diffusion analysis.
25. R Core Team. 2018 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See https://www.R-project.org/
26. Cairns SJ, Schwager SJ. 1987 A comparison of association indices. *Anim. Behav.* **35**, 1454–1469. (doi:10.1016/S0003-3472(87)80018-0)
27. Farine DR. 2013 Animal social network inference and permutations for ecologists in R using asnipe. *Methods Ecol. Evol.* **4**, 1187–1194. (doi:10.1111/2041-210X.12121)
28. Calenge C. 2015 Home range estimation in R: the adehabitatHR Package. 1–60. (doi:10.1111/j.1365-2656.2006.01186.x)
29. Fieberg J, Kochanny CO. 2005 Quantifying home-range overlap: the importance of the utilization distribution. *J. Wildl. Manage.* **69**, 1346–1359. (doi:10.2193/0022-541X(2005)69[1346:QHOTIO]2.0.CO;2)
30. Wild S, Hoppitt W. 2018 Choosing a sensible cut-off point: assessing the impact of uncertainty in a social network on the performance of NBDA. *Primates* **60**, 307–315. (doi:10.1007/s10329-018-0693-4))
31. Mann J, Sargeant BL, Watson-Capps JJ, Gibson QA, Heithaus MR, Connor RC, Patterson E. 2008 Why do dolphins carry sponges? *PLoS One* **3**, e3868. (doi:10.1371/journal.pone.0003868)
32. Sargeant BL, Wirsing AJ, Heithaus MR, Mann J. 2007 Can environmental heterogeneity explain individual foraging variation in wild bottlenose dolphins (*Tursiops* sp.)? *Behav. Ecol. Sociobiol.* **61**, 679–688. (doi:10.1007/s00265-006-0296-8)
33. Burnham K, Anderson D. 2002 *Model selection and multi-model inference: a practical information-theoretic approach*, 2nd edn. New York, NY: Springer.
34. Tyne J, Loneragan N, Kopps A, Allen S, Krützen M, Bejder L. 2012 Ecological characteristics contribute to sponge distribution and tool use in bottlenose dolphins *Tursiops* sp. *Mar. Ecol. Prog. Ser.* **444**, 143–153. (doi:10.3354/meps09410)
35. Mann J, Sargeant B. 2003 Like mother, like calf: the ontogeny of foraging traditions in wild Indian ocean bottlenose dolphins (*Tursiops* sp.). In *The biology of traditions: models and evidence* (eds D Fragaszy, S Perry), pp. 236–266. Cambridge, UK: Cambridge University Press.
36. Connor RC, Smolker RA, Richards AF. 1992 Two levels of alliance formation among male bottlenose dolphins (*Tursiops* sp.). *Proc. Natl Acad. Sci. USA* **89**, 987–990. (doi:10.1073/pnas.89.3.987)
37. Connor RC, Krützen M. 2015 Male dolphin alliances in Shark Bay: changing perspectives in a 30-year study. *Anim. Behav.* **103**, 223–235. (doi:10.1016/j.anbehav.2015.02.019)
38. Bizzozzero MR, Allen SJ, Gerber L, Wild S, King SL, Connor RC, Friedman WR, Wittwer S, Krützen M. 2019 Tool use and social homophily among male bottlenose dolphins. *Proc. R. Soc. B* **286**, 20190898. (doi:10.1098/rspb.2019.0898)
39. Patterson EM, Krzyszczyk E, Mann J. 2015 Age-specific foraging performance and reproduction in

tool-using wild bottlenose dolphins. *Behav. Ecol.* **27**, 401–410. (doi:10.1093/beheco/arv164)
40. Gibson QA, Mann J. 2008 Early social development in wild bottlenose dolphins: sex differences, individual variation and maternal influence. *Anim. Behav.* **76**, 375–387. (doi:10.1016/j.anbehav.2008.01.021)
41. Krzyszczyk E, Patterson EM, Stanton MA, Mann J. 2017 The transition to independence: sex differences in social and behavioural development of wild bottlenose dolphins. *Anim. Behav.* **129**, 43–59. (doi:10.1016/j.anbehav.2017.04.011)
42. Zefferman MR. 2016 Mothers teach daughters because daughters teach granddaughters: the evolution of sex-biased transmission. *Behav. Ecol.* **27**, 1172–1181. (doi:10.1093/beheco/arw022)
43. Mann J, Patterson E. 2013 Tool use by aquatic animals. *Phil. Trans. R. Soc. B* **368**, 20120424. (doi:10.1098/rstb.2012.0424)
44. Bacher K, Allen S, Lindholm AK, Bejder L, Krützen M. 2010 Genes or culture: are mitochondrial genes associated with tool use in bottlenose dolphins (*Tursiops* sp.)? *Behav. Genet.* **40**, 706–714. (doi:10.1007/s10519-010-9375-8)
45. Kopps AM, Ackermann CY, Sherwin WB, Allen SJ, Bejder L, Krützen M. 2014 Cultural transmission of tool use combined with habitat specializations leads to fine-scale genetic structure in bottlenose dolphins. *Proc. R. Soc. B* **281**, 20133245. (doi:10.1098/rspb.2013.3245)
46. McElreath R, Strimling P. 2008 When natural selection favors imitation of parents. *Curr. Anthropol.* **49**, 307–316. (doi:10.1086/524364)
47. Wild S, Allen SJ, Krützen M, King SL, Gerber L, Hoppitt WJE. 2019 Data from: Multi-network-based diffusion analysis reveals vertical cultural transmission of sponge tool use within dolphin matrilines. Dryad Digital Repository. (https://doi.org/10.5061/dryad.sc26m6c)

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Tool use and social homophily among male bottlenose dolphins

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Homophilous behaviour plays a central role in the formation of human friendships. Individuals form social ties with others that show similar phenotypic traits, independently of relatedness. Evidence of such homophily can be found in bottlenose dolphins (*Tursiops aduncus*) in Shark Bay, Western Australia, where females that use marine sponges as foraging tools often associate with other females that use sponges. 'Sponging' is a socially learned, time-consuming behaviour, transmitted from mother to calf. Previous research illustrated a strong female bias in adopting this technique. The lower propensity for males to engage in sponging may be due to its incompatibility with adult male-specific behaviours, particularly the formation of multi-level alliances. However, the link between sponging and male behaviour has never been formally tested. Here, we show that male spongers associated significantly more often with other male spongers irrespective of their level of relatedness. Male spongers spent significantly more time foraging, and less time resting and travelling, than did male non-spongers. Interestingly, we found no difference in time spent socializing. Our study provides novel insights into the relationship between tool use and activity budgets of male dolphins, and indicates social homophily in the second-order alliance composition of tool-using bottlenose dolphins.

1. Introduction

Individuals acquire information and behavioural skills from conspecifics through social learning across a variety of taxa, including insects, fishes, reptiles, birds and mammals [1–4]. Despite the widespread prevalence of social learning, this strategy may not always be beneficial, as knowledge gained from conspecifics can be maladaptive with one's own behavioural patterns [5]. It is therefore important for individuals to learn selectively from others to maximize benefits [6]. Explanations for *why*, *when* and *from whom* individuals learn include adopting behaviour performed by the majority [7], behaviour performed by kin [8] or based on increased pay-offs [9], among others (reviewed in [4,10]). However, while social learning has received considerable attention in the literature, relatively little is known about what differences exist between the sexes and what consequences such differences might hold for adult life.

Sexual selection theory predicts that males should primarily engage in behaviours related to increasing mating opportunities, while females should invest more in behaviours related to increasing access to resources and offspring protection [11,12]. Differences in behavioural requirements or preferences are therefore expected to dictate sex biases in social learning. For example, both

male and female chimpanzees (*Pan troglodytes*) learn socially to insert flexible tools made from vegetation into termite mounds in order to extract termites, yet females learn 'termite fishing' earlier, use it more frequently and do so more efficiently than males [13,14]. The differing priorities in learning to use a tool are reflective of the different strategies of male and female chimpanzees to maximize fitness. Chimpanzees use tools in foraging contexts; thus, the benefits of engaging in such a technique should be higher for females than males. Male chimpanzees form coalitions to compete for and maintain alpha male status, a social position that confers increased reproductive opportunity [15]. Consequently, males might be less inclined to invest in learning or improving complicated feeding techniques, but rather invest in social relationships with other males [16].

In the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) population of Shark Bay, Western Australia, sex bias is evident in a socially learned foraging technique involving the use of marine sponges as tools [17,18]. Sponge-carrying (sponging) is thought to protect the dolphin's rostrum while foraging for prey on the sea floor [17,19]. Sponging allows these dolphins (spongers) to exploit a novel ecological niche by providing access to prey not available to those dolphins unfamiliar with tool use [20]. Sponging is observed in both the eastern and western gulfs of Shark Bay, but only some members of particular matrilines use sponges (west: approx. 38% of all females [21]; east: approx. 13% of all females [22]). This is why sponging is thought to be an exclusively vertically transmitted behaviour [18,23]. Around 91% of female calves adopt sponging from their sponging mothers, while only 50% of males do so. The observed female bias in sponging is most likely to be reflective of a sex bias in social learning propensities at a young age [24–26].

Sponging females are distinctive with regard to their activity budget, spending more time foraging and less time resting than their non-sponging female counterparts [21,24]. When foraging, female spongers devote 95% of their time to sponging, compared with other foraging behaviours [24]. They are also seen alone more often than non-spongers [22,24]. However, when associating with other individuals, female spongers show a preference for other sponging females [22]. While there is a considerable amount of data on female spongers, much less is known about male spongers. For instance, why proportionally fewer males learn and specialize in this foraging technique, and if and how sponging influences adult male behaviour, remain unknown. The latter is of particular relevance as male dolphins in Shark Bay exhibit one of the most complex social structures outside humans (reviewed in [27]).

Bottlenose dolphins in Shark Bay live in an open fission–fusion society with changing group sizes and compositions [27,28]. Males form different levels of reproductive alliances with other males, driven by intense competition for access to receptive females [27]. Two to three males cooperate in 'first-order' alliances to consort single oestrus females [29]. These males also generally associate within larger 'second-order' alliances composed of 4–14 individuals, whose members cooperate to take females from rival alliances and to defend against such attacks [29]. First- and second-order allies are also frequently observed together in non-mating contexts [29]. Second-order alliances are considered the stable, core unit of male social organization in Shark Bay, while the stability of first-order alliances varies considerably [27]. These complex social relationships among males can last for decades and are critical to each male's reproductive success [27]. Alliances are considered costly, as each male must invest time in the formation and maintenance of these relationships [30].

Sponging is also a costly behaviour: it requires significant time investment and is associated with a decrease in overall sociability [22,24], as well as less time to rest and travel [21]. The investment of time and energy into male alliance behaviours may therefore preclude engaging in time-consuming, solitary foraging techniques, such as sponging. It has been proposed that sponging might put males at a disadvantage in forming and maintaining alliances compared with males that use foraging techniques that are both less time-consuming and less solitary [17,18,21,24]. However, these arguments assume that the time, social demands and energetic demands of sponging on males and females are similar, which has yet to be tested. Here, we assess the effect of sponging on male dolphin behaviour by comparing activity budgets, sociability and association patterns of male spongers to male non-spongers.

2. Methods

(a) Study site and data collection

Data for this study were collected in the western gulf of Shark Bay, Western Australia, in an area that includes various habitat types, such as seagrass-rich shallow waters (less than 10 m) and deep-water channels with sandy substrates (greater than 10 m) [31]. We collected behavioural and genetic data during the austral winters from 2007 to 2015, identifying individual dolphins by photographs of their dorsal fins [32]. During boat-based surveys of dolphin groups, within the first 5 min, we recorded GPS position, environmental parameters (including sea state, water depth and temperature), group size and composition, as well as predominant group activity (rest, travel, forage, socialize or unknown; cf. [33] and electronic supplementary material). We defined group membership according to the 10 m chain rule [33]. Male dolphins that had been observed carrying a sponge while foraging at least twice on different days were classified as spongers [24], while males that had never been observed sponging were classified as non-spongers. Individuals that had been observed sponging only once were classified as 'unknowns'. We obtained biopsy samples from dolphins on an opportunistic basis using a purpose-designed system for sampling small cetaceans [34]. The samples were used to genetically sex individuals [35] and determine pairwise genetic relatedness [18]. Further details of sampling and laboratory methods are provided in the electronic supplementary material. Unless otherwise specified, all analyses were conducted in R v. 1.1.453 [36].

(b) Data restriction

We included only independent/weaned males and excluded dependent calves [37]. Only males observed more than nine times and identified as spongers or non-spongers were included in our analyses. Sex was identified either genetically (see electronic supplementary material) or behaviourally by several observations of alliance-typical behaviour (being observed regularly travelling side-by-side engaging in synchronous surfacing, consorting of females or inter-group aggression with other males; cf. [27,38]). Furthermore, in order to assess males with similar association opportunities, we restricted our analyses to comparisons of male spongers with non-sponging males that also met habitat use criteria based on depth and home range overlap derived from data on sponging males. Further details

on the calculation of these criteria are provided in the electronic supplementary material. Restricting the data in this manner resulted in a dataset containing 37 male dolphins, including 13 spongers and 24 non-spongers.

(c) Effect of sponging on male activity budgets

To investigate differences in activity budgets (proportions of resting, travelling, foraging and socializing behaviour) between male spongers and non-spongers, we conducted a multivariate analysis of variance (MANOVA) with the sole predictor of whether an individual was classified as sponger or non-sponger (hereafter: foraging technique). As dependent variables, we calculated activity budgets by dividing the number of individual sightings per activity by the total number of individual sightings. We used Pillai's trace (V) as a test statistic due to the unequal sample sizes in our dataset [39]. To investigate which activity proportions, in particular, differed between male spongers and non-spongers, we performed sequential Bonferroni-corrected, post hoc, independent t-tests (Welch's t-test [40]). While investigating the data structure of the multivariate activity budgets, we identified five outliers from the combined normal distribution. Thus, we conducted the MANOVA with outliers removed, retaining 32 males (spongers: $n = 12$, non-spongers: $n = 20$) in the dataset (see electronic supplementary material for analysis with the full dataset).

(d) Degree of sociability of male spongers and non-spongers

To investigate whether male spongers were more or less solitary than male non-spongers, we compared their levels of sociability. We constructed an index of sociability by dividing the number of solitary sightings by the total number of sightings per individual. We compared individual sociability indices of male spongers and male non-spongers in a two-sample permutation test (10 000 permutations) implemented in the 'perm' package [41].

To investigate the association pattern of male spongers and male non-spongers, we adhered to the following procedure. First, to maximize our ability to draw comparisons with other studies on cetaceans, we calculated half weight indices (HWIs) as a measure of the proportion of time two males spent together [42]. Based on the dyadic HWIs, we created a social network to analyse the association patterns between male spongers and male non-spongers. Second, we assessed whether associations in the social network followed a random pattern or whether two individuals were seen more or less often together than expected by chance [43,44]. For this analysis, we specified a daily sampling period. Third, to test whether the association indices between pairs consisting of males with similar foraging techniques (sponger–sponger; non-sponger–non-sponger) were higher than between pairs with different foraging techniques (sponger–non-sponger), we carried out a Mantel test on a similarity matrix and the matrix of dyadic associations with 10 000 permutations. The similarity matrix is a 1/0 matrix providing information on whether two individuals belong to the same group (either both spongers or both non-spongers = 1) or to different groups (sponger and non-sponger = 0). These analyses were conducted in SOCPROG 2.6 [45].

In a further step, we ran a double decker semi-partialling multiple regression quadratic assignment procedure (MRQAP-DSP; see below and [46]) to investigate whether the documented pattern of dyadic associations (between male pairs of spongers, pairs of non-spongers, and pairs of one sponger and one non-sponger) could be predicted by similarity in foraging technique, even when controlling for pairwise relatedness (based on 27 microsatellite loci; see electronic supplementary material for more detailed information). Similarity in foraging technique was presented in two matrices: in the first, we coded similarity in sponging as 1; and vice versa in the second, where similarity in non-sponging was coded as 1. Unequal dyads were assigned a value of 0 in both matrices. Separate similarity matrices allowed us to disentangle the contribution of similarity in sponging and non-sponging, respectively, to the association pattern.

An MRQAP-DSP test is similar to a partial linear multiple regression with the exception that dependent and predictor variables are presented as matrices. Thus, this method tests whether an entered predictor variable significantly contributes to the explanation of the dependent matrix, while controlling for the other predictors. To control for the dependencies between data points, we used the MRQAP-DSP test as implemented and described in the 'asnipe' package [47] using 10 000 permutations. We did not include mitochondrial haplotypes in the predictors due to a previously documented high correlation with foraging technique [48]. Only males for which we had genetic data available were included in this test (spongers: $n = 9$, non-spongers: $n = 16$). We also repeated the MRQAP-DSP test including all genotyped males within our study population while additionaly correcting for home range overlaps (see electronic supplementary material).

To investigate whether the association patterns found in the previous analysis were also reflected in second-order alliance compositions, we defined second-order alliances based on dyadic HWIs. We lacked sufficient consortship data to define alliances functionally (i.e. through observation of consortship behaviour) for this study, so we could use only association strength as a proxy [33]. We used an average linkage agglomerative cluster analysis assuming a hierarchical social network structure [49] performed in SOCPROG [45], and defined and applied a threshold value at which a dyad can be considered to be part of the same second-order alliance. To find an appropriate threshold, we conducted a change point analysis employing the pruned exact linear time (PELT) method specified in the 'changepoint' package [50] (cf. [51] and electronic supplementary material for more detailed information).

3. Results

Between 2007 and 2015, we observed 124 male dolphins at least nine times. After applying the restrictions outlined above, the resulting dataset contained 37 male dolphins, of which 13 were spongers and 24 were non-spongers (number of sightings: mean = 35; range = 17–68). We computed HWIs from a total of 549 survey records over the 9-year study period. All males associated with at least five other individuals in the dataset.

(a) Effect of sponging on male activity budgets

We detected significantly different activity budgets between male spongers and non-spongers ($V = 0.74$, $F_{4,27} = 19.6$, $p < 0.001$). Thus, foraging techniques significantly contributed to explaining an individual male's activity budget. Post hoc analyses showed that male spongers foraged more, and rested and travelled less than male non-spongers. There was no significant difference in time spent socializing between male spongers and non-spongers (table 1).

(b) Degree of sociability of male spongers and male non-spongers

Male spongers were encountered significantly more often alone (sociability index: mean = 0.22, s.e. = 0.03) than

Table 1. Post hoc, Bonferroni-corrected *t*-tests on activity proportions of male spongers ($n = 12$) and non-spongers ($n = 20$). Significant *p*-values are indicated in italics.

	spongers		non-spongers				
proportion	mean	s.d.	mean	s.d.	*t* (d.f.)	*r*	*p*-value
forage	0.45	0.02	0.20	0.02	−9.42 (26.31)	0.89	*<0.001*
rest	0.18	0.01	0.28	0.01	4.83 (27.80)	0.68	*<0.001*
travel	0.16	0.02	0.31	0.02	4.83 (27.36)	0.68	*<0.001*
socialize	0.16	0.01	0.13	0.01	−1.62 (29.99)	0.28	0.23

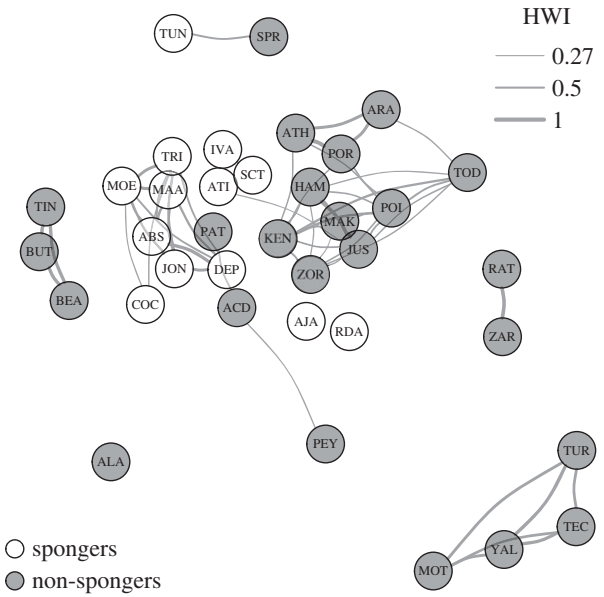

Figure 1. Social network of the male dolphins in the restricted dataset ($n = 37$). The nodes represent individuals and are shaded according to foraging technique. Edges (lines) below 0.27 HWI are transparent and edge thickness corresponds to edge weight (see electronic supplementary material, figure S2 for the social network showing all edges). The graph was plotted with the force-directed Fruchterman–Reingold algorithm implemented in the 'igraph' package [52].

male non-spongers (sociability index: mean = 0.04, s.e. = 0.01; $p = 0.002$).

Among the 37 males, the overall mean HWI was 0.09 (1000 bootstraps: s.e. = 0.03), including the zeros of no associations. Considering only non-zero associations, the more conservative measure, the mean HWI was 0.17 (1000 bootstraps: s.e. = 0.05). The generated network based on the dyadic association indices (figure 1) represented a non-random social structure (10 000 permutations, 1000 switches; $\text{s.d.}_{\text{obs}} = 0.17$, $\text{s.d.}_{\text{random}} = 0.14$, $p < 0.001$). Thus, some males were observed more often in association than expected by chance alone, reflecting their well-documented alliance associations [27].

Association rates between pairs of males with similar foraging techniques (sponger–sponger; non-sponger–non-sponger; mean HWI = 0.14, s.d. = 0.09) were significantly higher (Mantel test, $t = 5.75$; $p < 0.01$; table 2) than associations between pairs with different foraging techniques (sponger–non-sponger: mean HWI = 0.05, s.d. = 0.04).

The MRQAP regression model showed that sponging was a significant predictor of male association patterns, even after controlling for relatedness (table 3). Related individuals did not associate above chance levels. These findings were also supported by the results of the MRQAP-DSP tests including all males within our study area (see electronic supplementary material for more information). Our analyses demonstrate that the association pattern of male dolphins inhabiting deep water and occupying similar home ranges can at least partly be explained by foraging technique.

Table 2. Mean association indices (HWI) by foraging technique of male spongers ($n = 13$) and non-spongers ($n = 24$), 666 dyadic relationships.

pair composition	mean HWI (s.d.)
sponger–sponger	0.21 (0.11)
non-sponger–non-sponger	0.10 (0.05)
similar foraging technique	0.14 (0.09)
different foraging technique	0.05 (0.04)
overall	0.09 (0.04)

Table 3. MRQAP-DSP model including only genotyped males ($n = 25$; 300 dyadic relationships). Significant *p*-values are indicated in italics.

variable	coefficient	*p*-value
sponger similarity	0.19	*<0.001*
non-sponger similarity	0.10	*<0.01*
relatedness	0.21	0.24
$F_{3,297} = 34.5$, adjusted $R^2 = 0.25$, *p*-value *<0.001*		

An average linkage agglomerative cluster analysis to define second-order alliances resulted in a tree diagram representing the underlying data well with a cophenetic correlation coefficient of 0.98 [45,53]. The PELT method resulted in a change point at HWI ≥ 0.27. This cut-off value is higher but well within the range of previous findings on the male dolphins of Shark Bay, in which an HWI of 0.20 has commonly been used in assigning males to second-order alliances [27,33]. Applying 0.27 as a threshold to define second-order alliances illustrated that the tendency of male spongers to associate with other male spongers was reflected in second-order alliance compositions. We identified nine second-order alliances, of which two consisted exclusively of spongers, one was of mixed composition (sponger and non-sponger) and the other six were composed

exclusively of non-spongers (figure 2). Four individuals (three spongers, one non-sponger) could not be assigned to a second-order alliance. Five of the non-sponging alliances and both sponging alliances have also been observed engaging in functional alliance behaviour (e.g. consorting females). A similar pattern was found when we included all males in our study population (see electronic supplementary material for more detail).

4. Discussion

It has been hypothesized that the investment of time and energy into the formation and maintenance of male alliances probably reduces the propensity for male dolphins to engage in time-consuming, solitary foraging techniques such as sponging, thereby resulting in the strong female bias previously documented [17,18,21,24]. This hypothesis was based on the assumptions that male spongers engage in different activity and social patterns than male non-spongers. Our results support these assumptions by revealing that, at least in the austral winters when data were collected, male spongers differed in their activity budgets, foraging more, and resting and travelling less, than male non-spongers. Interestingly, the time spent socializing was equal among male spongers and non-spongers despite the fact that male spongers spent more time alone than male non-spongers. When male spongers were observed with other males, they associated significantly more often with other male spongers.

Previous studies on female activity budgets in Shark Bay also found that spongers spent a greater proportion of their time foraging and less time resting and travelling than their non-sponging counterparts [21,24], suggesting that time investment could be a proximate cost of sponging in comparison with other foraging techniques for both sexes. A comparison between the sexes warrants further investigation. Interestingly, socializing proportions for males seem not to be affected by these time investments, suggesting that a comparatively smaller amount of time spent resting might be the proximate cost of sponging. However, these potential costs might be offset by having fewer competitors for food, as sponging may decrease competition for resources by providing access to a novel ecological niche [19,20]. Indeed, the role of intraspecific competition on niche expansion has been reported across several taxa [54,55].

Our finding that male spongers and male non-spongers spent equal amounts of time socializing contradicts the hypothesis that sponging conflicts with cooperative male alliance behaviour. However, when comparing sociability, we found that male spongers had higher proportions of solitary sightings compared with male non-spongers. Our findings thereby corroborate previous studies indicating that sponging is a largely solitary activity [21,24]. The increased solitariness of male spongers might still affect cooperative male alliance behaviour negatively to some degree, even though there is no difference in socializing time.

Our examination of male social structure in deep-water habitat revealed that male spongers tended to associate with other male spongers rather than male non-spongers, as demonstrated by their clustering in the social network. Sponging was a significant predictor of the observed association patterns of males sharing similar home ranges even after controlling for pairwise relatedness and similarity in non-sponging. Likewise, when we repeated our analysis and included all genotyped males, similarity in sponging remained a significant predictor for social structuring (see electronic supplementary material for more information). These results contradict a previous study on male dolphins in eastern Shark Bay [22], which did not detect a significant effect of similarity in foraging technique on social structuring. This was most likely to be a result of low sample size as there are far fewer spongers, and particularly male spongers, in the eastern gulf of Shark Bay compared with the western gulf [22,31]. Remarkably, in our study, while similarity in foraging technique was significant in terms of impact on social structuring, pairwise relatedness was not (table 3). The absence of an effect of relatedness on the social structuring of male dolphins seems plausible; previous studies on male associations and relatedness of second-order alliances reported ambiguous patterns, with only a minority of alliances showing higher relatedness than the population average [56].

The high social affinity among male spongers could either indicate social learning of tool use from alliance partners or be explained by homophilous behaviour (i.e. increased associations due to similar behaviour). The established pattern of strict vertical transition of sponging [18,23] and the reported homophily related to sponging in female dolphins of Shark Bay [22] make homophily among male spongers the more parsimonious explanation. Whether the observed homophily among male spongers is driven by the males themselves or emerges as a by-product of the high social affinity of female spongers (i.e. mothers) remains unknown. Research in eastern

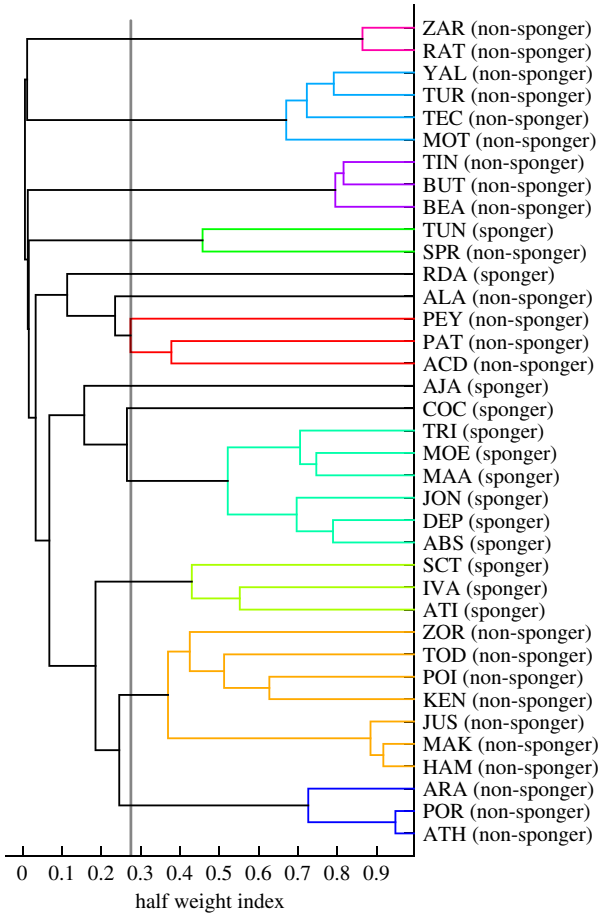

Figure 2. Hierarchical cluster diagram based on dyadic HWI measures. An HWI value of 0.27 was used as a cut-off value (grey line) to define communities (i.e. second-order alliances).

Shark Bay has shown that juvenile males preferentially stayed in proximity to their natal associates [57], and the number of associates stays constant from infancy through the juvenile period [58]. If the natal associates of spongers were also male spongers, this could explain the high social bonds between pairs or trios of sponging males. As sponging females—and hence, mothers of sponging males—are shown to cluster together [22], such a scenario seems plausible.

The ultimate benefit of such homophilous behaviour in male spongers could be their ability to maintain the use of such a foraging technique while simultaneously remaining in close proximity to males 'of a similar ilk' (i.e. with whom they can also engage in alliance behaviours). This argument is further strengthened when considering the composition of second-order alliances. There was only one mixed second-order alliance, while the other eight alliances in our dataset consisted of either only male spongers or male non-spongers. The threshold resulting from our PELT analysis to identify second-order alliances was higher than previously documented in Shark Bay [29], resulting in the delineation of a greater number of alliances, with some having fewer members than typically reported for second-order alliances [27,29]. The higher threshold of 0.27 may have split some second-order alliances that associated at levels of greater than 0.20 but less than 0.27. Thus, the smaller second-order alliances identified in our study comprising only two to three males are probably first-order allies. Yet, irrespective of the threshold used to define alliances, when considering the hierarchical structure of the social network (i.e. dyadic associations assorted in a dendrogram, figure 2), social homophily is apparent. Given the need to synchronize activities when living in groups (i.e. in alliances) [59], males in alliances containing sponging and non-sponging individuals might be at a disadvantage relative to non-mixed alliances. Future research needs to examine whether there are differences in the structure and complexity of second- and first-order alliances between male spongers and non-spongers. Here, we suggest that the benefits of social homophily may, to a certain extent, mitigate the costs of sponging for male alliance behaviour.

Apart from social homophily, behavioural plasticity might manifest itself by allied male spongers reducing the amount of time invested in sponging during the peak mating season, thus further mitigating the costs of being a male sponger to some degree. Nevertheless, the mating season in Shark Bay is only moderately seasonal, with consortships occurring during all months of the year, and a diffuse peak between September and December [60].

In summary, we show that while previous assumptions that sponging affects male activity budgets and social pattern hold true, this might not necessarily stand in conflict with male alliance behaviour. The apparent cost-mitigating behaviours together with the observed absence of differences in socializing proportions between male spongers and non-spongers weaken the hypothesis that sponging stands in conflict with male alliance behaviour, thereby leading to a female bias in sponging. In fact, preliminary data suggest rates of female monopolization do not differ between male spongers and male non-spongers (M.R.B. 2016, unpublished data). Future research might explore the costs of sponging and how it might be mitigated in more detail, leaving room for other plausible explanations regarding female bias in social learning of sponging. For instance, time constraints on a male dolphin during its early life may play an important role. Males are weaned earlier than females [61], and therefore have less time to learn sponging from their mothers; instead, they may need to invest time in developing social bonds with other males. Indeed, juvenile male dolphins invest more time in developing social skills than juvenile females, who instead increase their foraging rates [58]. In addition, a recent study showed that an extensive training period (decades) is crucial to achieve peak performance in sponging [26].

In conclusion, our study explored the impacts of sponging on male dolphin behaviour. We suggest that potential costs associated with sponging for male dolphins might be mitigated by social homophily. Revealing social homophily in bottlenose dolphins is interesting, as in humans, homophilous behaviour is a key factor in the emergence and maintenance of subcultures [62], and the establishment of attachment and close friendships [63]. Our study thereby provides another example of convergence in social complexity, innovation and cultural behaviour between cetaceans and great apes [20,22,64,65].

Data accessibility. All used datasets are available as electronic supplementary material to this study.

Authors' contributions. Conceived and designed study: M.K., S.J.A. and M.R.B. Carried out field and laboratory work: S.J.A., L.G., S.Wil., S.L.K., M.K., S.Wit., W.R.F., R.C.C. and M.R.B. Performed statistical analyses: M.R.B. Wrote the manuscript: M.R.B., S.J.A. and M.K. Edited the manuscript: S.L.K., L.G., S.Wil., R.C.C., W.R.F. and S.Wit.

Competing interests. We declare we have no competing interests.

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References

1. Laland K, Janik V. 2006 The animal cultures debate. *Trends Ecol. Evol.* **21**, 542–547. (doi:10.1016/j.tree.2006.06.005)
2. Leadbeater E, Chittka L. 2007 Social learning in insects—from miniature brains to consensus building. *Curr. Biol.* **17**, R703–R713. (doi:10.1016/j.cub.2007.06.012)
3. Hoppitt W, Laland KN. 2008 Social processes influencing learning in animals: a review of the evidence. *Adv. Stud. Behav.* **38**, 105–165. (doi:10.1016/S0065-3454(08)00003-X)
4. Fogarty L, Laland KN, Morgan TJH, Webster MM, Hoppitt WJE, Rendell L. 2011 Cognitive culture: theoretical and empirical insights into social learning strategies. *Trends Cogn. Sci.* **15**, 68–76. (doi:10.1016/j.tics.2010.12.002)
5. Giraldeau L, Valone TJ, Templeton JJ. 2002 Potential disadvantages of using socially acquired information. *Phil. Trans. R Soc. Lond. B* **357**, 1559–1566. (doi:10.1098/rstb.2002.1065)

6. Laland KN. 2011 Social learning strategies. *Anim. Learn. Behav.* **32**, 4–14. (doi:10.3758/bf03196002)
7. Pike TW, Laland KN. 2010 Conformist learning in nine-spined sticklebacks' foraging decisions. *Biol. Lett.* **6**, 466–468. (doi:10.1098/rsbl.2009.1014)
8. Henrich J, Henrich N. 2010 The evolution of cultural adaptations: Fijian food taboos protect against dangerous marine toxins. *Proc. R. Soc. B* **277**, 3715–3724. (doi:10.1098/rspb.2010.1191)
9. Kendal JR, Rendell L, Pike TW, Laland KN. 2009 Nine-spined sticklebacks deploy a hill-climbing social learning strategy. *Behav. Ecol.* **20**, 238–244. (doi:10.1093/beheco/arp016)
10. Hoppitt W, Laland KN. 2013 *Social learning: an introduction to mechanisms, methods, and models*. Princeton, NJ: Princeton University Press.
11. Trivers RL. 1972 Parental investment and sexual selection. In *Sexual selection and the descent of man 1871–1971* (ed. B Campbell), pp. 136–179. Chicago, IL: Aldine.
12. Bateman AJ. 1984 Intra-sexual selection in Drosophila. *Heredity (Edinb)* **2**, 349–368.
13. Lonsdorf EV. 2005 Sex differences in the development of termite-fishing skills in the wild chimpanzees, *Pan troglodytes schweinfurthii*, of Gombe National Park, Tanzania. *Anim. Behav.* **70**, 673–683. (doi:10.1016/j.anbehav.2004.12.014)
14. Lonsdorf EV, Anderson KE, Stanton MA, Shender M, Heintz MR, Goodall J *et al.* 2014 Boys will be boys: sex differences in wild infant chimpanzee social interactions. *Anim. Behav.* **88**, 79–83. (doi:10.1016/j.anbehav.2013.11.015%0A)
15. Wroblewski EE, Murray CM, Keele BF, Schumacher-Stankey JC, Hahn BH, Pusey AE. 2009 Male dominance rank and reproductive success in chimpanzees, *Pan troglodytes schweinfurthii*. *Anim. Behav.* **77**, 873–885. (doi:10.1016/j.anbehav.2008.12.014)
16. Gilby IC, Brent LJN, Wroblewski EE, Rudicell RS, Hahn BH, Goodall J, Pusey AE. 2013 Fitness benefits of coalitionary aggression in male chimpanzees. *Behav. Ecol. Sociobiol.* **67**, 373–381. (doi:10.1007/s00265-012-1457-6)
17. Smolker R, Richards A, Connor R, Mann J, Berggren P. 1997 Sponge carrying by dolphins (Delphinidae, *Tursiops* sp.): a foraging specialization involving tool use? *Ethology* **103**, 454–465. (doi:10.1111/j.1439-0310.1997.tb00160.x)
18. Krützen M, Mann J, Heithaus MR, Connor RC, Bejder L, Sherwin WB. 2005 Cultural transmission of tool use in bottlenose dolphins. *Proc. Natl Acad. Sci. USA* **102**, 8939–8943. (doi:10.1073/pnas.0500232102)
19. Patterson EM, Mann J. 2011 The ecological conditions that favor tool use and innovation in wild bottlenose dolphins (*Tursiops* sp.). *PLoS ONE* **6**, e22243. (doi:10.1371/journal.pone.0022243)
20. Krützen M, Kreicker S, MacLeod CD, Learmonth J, Kopps AM, Walsham P, Allen SJ. 2014 Cultural transmission of tool use by Indo-Pacific bottlenose dolphins (*Tursiops* sp.) provides access to a novel foraging niche. *Proc. R. Soc. B* **281**. 20140374. (doi:10.1098/rspb.2014.0374)
21. Kopps AM, Krützen M, Allen SJ, Bacher K, Sherwin WB. 2014 Characterizing the socially transmitted foraging tactic 'sponging' by bottlenose dolphins (*Tursiops* sp.) in the western gulf of Shark Bay, Western Australia. *Mar. Mammal Sci.* **30**, 847–863. (doi:10.1111/mms.12089)
22. Mann J, Stanton MA, Patterson EM, Bienenstock EJ, Singh LO. 2012 Social networks reveal cultural behaviour in tool-using using dolphins. *Nat. Commun.* **3**, 980. (doi:10.1038/ncomms1983)
23. Bacher K, Allen S, Lindholm AK, Bejder L, Krützen M. 2010 Genes or culture: are mitochondrial genes associated with tool use in bottlenose dolphins (*Tursiops* sp.)? *Behav. Genet.* **40**, 706–714. (doi:10.1007/s10519-010-9375-8)
24. Mann J, Sargeant BL, Watson-Capps JJ, Gibson QA, Heithaus MR, Connor RC, Patterson E. 2008 Why do dolphins carry sponges? *PLoS ONE* **3**, e3868. (doi:10.1371/journal.pone.0003868)
25. Mann J, Patterson EM. 2013 Tool use by aquatic animals. *Phil. Trans. R. Soc. B* **368**, 20120424. (doi:10.1098/rstb.2012.0424)
26. Patterson EM, Krzyszczyk E, Mann J. 2016 Age-specific foraging performance and reproduction in tool-using wild bottlenose dolphins. *Behav. Ecol.* **27**, 401–410. (doi:10.1093/beheco/arv164)
27. Connor RC, Krützen M. 2015 Male dolphin alliances in Shark Bay: changing perspectives in a 30-year study. *Anim. Behav.* **103**, 223–235. (doi:10.1016/j.anbehav.2015.02.019)
28. Connor RC, Wells RJ, Mann J, Read A. 2000 The bottlenose dolphin: social relationships in a fission–fusion society. In *Cetacean societies: field studies of dolphins and whales* (eds J Mann, R Connor, P Tyack, H Whitehead), pp. 91–126. Chicago, IL: University of Chicago Press.
29. Connor RC, Smolker RA, Richards AF. 1992 Two levels of alliance formation among male bottlenose dolphins (*Tursiops* sp). *Proc. Natl Acad. Sci. USA* **89**, 987–990. (doi:10.1073/pnas.89.3.987)
30. Gerber L *et al*. In review. Multi-level cooperation in wild male bottlenose dolphins is predicted by long-term friendships. *Behav. Ecol.*
31. Tyne JA, Loneragan NR, Kopps AM, Allen SJ, Krützen M, Bejder L. 2012 Ecological characteristics contribute to sponge distribution and tool use in bottlenose dolphins *Tursiops* sp. *Mar. Ecol. Prog. Ser.* **444**, 143–153. (doi:10.3354/meps09410)
32. Würsig B, Würsig M. 1977 The photographic determination of group size, composition, and stability of coastal porpoises (*Tursiops truncatus*). *Science* **198**, 755–756.
33. Smolker RA, Richards AF, Connor RC, Pepper JW. 1992 Sex differences in patterns of association among Indian Ocean bottlenose dolphins. *Behaviour* **123**, 38–69. (doi:10.1163/156853992X00101)
34. Krützen M, Barre L, Möller L, Heithaus M, Simms C, Sherwin W. 2002 A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Mar. Mammal Sci.* **18**, 863–878. (doi:10.1111/j.1748-7692.2002.tb01078.x)
35. Gilson A, Syvanen M, Levine K, Banks J. 1998 Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. *Calif. Fish Game* **84**, 159–169.
36. R Core Team. 2013 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See http://www.r-project.org
37. Mann J, Connor RC, Barré LM, Heithaus MR. 2000 Female reproductive success in bottlenose dolphins (*Tursiops* sp.): life history, habitat, provisioning, and group-size effects. *Behav. Ecol.* **11**, 210–219. (doi:10.1093/beheco/11.2.210)
38. Connor RC, Smolker R, Bejder L. 2006 Synchrony, social behaviour and alliance affiliation in Indian Ocean bottlenose dolphins, *Tursiops aduncus*. *Anim. Behav.* **72**, 1371–1378. (doi:10.1016/j.anbehav.2006.03.014)
39. Tabachnick BG, Fidell LS, Ullman JB. 2007 *Using multivariate statistics*. Boston, MA: Pearson.
40. Welch WJ. 1990 Construction of permutation tests. *J. Am. Stat. Assoc.* **85**, 693–698. (doi:10.1080/01621459.1990.10474929)
41. Fay M, Shaw PA. 2010 Exact and asymptotic weighted logrank tests for interval censored data: the interval R Package. *J. Stat. Softw.* **36**, 1–34.
42. Cairns SJ, Schwager SJ. 1987 A comparison of association indices. *Anim. Behav.* **35**, 1454–1469. (doi:10.1016/S0003-3472(87)80018-0)
43. Bejder L, Fletcher D, Bräger S. 1998 A method for testing association patterns of social animals. *Anim. Behav.* **56**, 719–725. (doi:10.1006/anbe.1998.0802)
44. Whitehead H, Bejder L, Ottensmeyer CA. 2005 Testing association patterns: issues arising and extensions. *Anim. Behav.* **69**, e1–e6. (doi:10.1016/j.anbehav.2004.11.004)
45. Whitehead H. 2009 SOCPROG: program for analyzing social structure. *Behav. Ecol. Sociobiol.* **63**, 765–778. (doi:10.1007/s00265-008-0697-y%0A)
46. Dekker D, Krackhardt D, Snijders TAB. 2007 Sensitivity of MRQAP tests to collinearity and autocorrelation conditions. *Psychometrika* **72**, 563–581. (doi:10.1007/s11336-007-9016-1)
47. Farine DR. 2013 Animal social network inference and permutations for ecologists in R using *asnipe*. *Methods Ecol. Evol.* **4**, 1187–1194. (doi:10.1111/2041-210X.12121)
48. Kopps AM, Ackermann CY, Sherwin WB, Allen SJ, Bejder L, Krützen M. 2014 Cultural transmission of tool use combined with habitat specializations leads to fine-scale genetic structure in bottlenose dolphins. *Proc. R. Soc. B* **281**, 20133245. (doi:10.1098/rspb.2013.3245)
49. Whitehead H. 2008 *Analyzing animal societies: quantitative methods for vertebrate social analysis*, pp. 161–168. Chicago, IL: University of Chicago Press. See http://public.eblib.com/choice/publicfullrecord.aspx?p=408184
50. Killick R, Haynes K, Eckley I, Fearnhead P, Lee J. 2016 'changepoint': methods for changepoint detection. See https://cran.r-project.org/web/packages/changepoint/changepoint.pdf
51. King SL *et al.* 2018 Bottlenose dolphins retain individual vocal labels in multi-level alliances.

Curr. Biol. **28**, 1993–1999. (doi:10.1016/j.cub.2018.05.013)

52. Csárdi G, Nepusz T. 2014 The igraph software package for complex network research. *J. Comput. Appl.* Complex Sy:9. See https://igraph.org/r.
53. Bridge PD, Fry J. 1993 Classification. In *Biological data analysis* (ed. J Fry), pp. 219–242. Oxford, UK: Oxford University Press.
54. Smith TB, Skúlason S. 1996 Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu. Rev. Ecol. Syst.* **27**, 111–133. (doi:10.1146/annurev.ecolsys.27.1.111)
55. Bolnick DI. 2001 Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. *Nature* **410**, 463–466. (doi:10.1038/35068555)
56. Krützen M, Sherwin WB, Connor RC, Barré LM, Van De Casteele T, Mann J, Brooks R. 2003 Contrasting relatedness patterns in bottlenose dolphins (*Tursiops* sp.) with different alliance strategies. *Proc. R. Soc. B* 497–502. (doi:10.1098/rspb.2002.2229)
57. Tsai YJJ, Mann J. 2012 Dispersal, philopatry, and the role of fission–fusion dynamics in bottlenose dolphins. *Mar. Mammal Sci.* **29**, 261–279. (doi:10.1111/j.1748-7692.2011.00559.x)
58. Krzyszczyk E, Patterson EM, Stanton MA, Mann J. 2017 The transition to independence: sex differences in social and behavioural development of wild bottlenose dolphins. *Anim. Behav.* **129**, 43–59. (doi:10.1016/j.anbehav.2017.04.011)
59. Tosi CH, Ferreira RG. 2010 Differences between solitary and group time budgets in Guiana dolphin (*Sotalia guianensis*) at northeastern Brazil. In *Whales and dolphins behavior, biology and distribution* (ed. CA Murray), pp. 139–150. New York, NY: Nova Science Publishers.
60. Smolker RA, Connor RC. 1996 'Pop' goes the dolphin: a vocalization male bottlenose dolphins produce during consortships. *Behaviour* **133**, 643–662. (doi:10.1163/156853996X00404)
61. Mann J, Sargeant BL. 2003 Like mother like calf: the ontogeny of foraging traditions in wild Indian Ocean bottlenose dolphins (*Tursiops* sp.). In *The biology of traditions* (eds DM Fragaszy, S Perry), pp. 236–266. Cambridge, UK: Cambridge University Press.
62. McPherson M, Smith-Lovin L, Cook JM. 2001 Birds of a feather: homophily in social networks. *Annu. Rev. Sociol.* **27**, 415–444. (doi:10.1146/annurev.soc.27.1.415)
63. Rivera MT, Soderstrom SB, Uzzi B. 2010 Dynamics of dyads in social networks: assortative, relational, and proximity mechanisms. *Annu. Rev. Sociol.* **36**, 91–115. (doi:10.1146/annurev.soc.34.040507.134743)
64. Allen SJ, King SL, Krützen M, Brown AM. 2017 Multi-modal sexual displays in Australian humpback dolphins. *Sci. Rep.* **7**, 13644. (doi:10.1038/s41598-017-13898-9)
65. Lonsdorf EV, Eberly LE, Pusey AE. 2004 Sex differences in learning in chimpanzees. *Nature* **428**, 715–716. (doi:10.1038/428715a)

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Long-term decline in survival and reproduction of dolphins following a marine heatwave

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One of many challenges in the conservation of biodiversity is the recent trend in the frequency and intensity of extreme climatic events [1]. The Shark Bay World Heritage Area, Western Australia, endured an unprecedented marine heatwave in 2011. Catastrophic losses of habitat-forming seagrass meadows followed [2], along with mass mortalities of invertebrate and fish communities [3]. Our long-term demographic data on Shark Bay's resident Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) population revealed a significant decline in female reproductive rates following the heatwave. Moreover, capture–recapture analyses indicated 5.9% and 12.2% post-heatwave declines in the survival of dolphins that use tools to forage and those that do not, respectively. This implies that the tool-using dolphins may have been somewhat buffered against the cascading effects of habitat loss following the heatwave by having access to a less severely affected foraging niche [4]. Overall, however, lower survival has persisted post-heatwave, suggesting that habitat loss following extreme weather events may have prolonged, negative impacts on even behaviourally flexible, higher-trophic level predators.

In the 2011 Austral summer, Western Australian coastal water temperatures rose 2–4°C above long-term averages for more than two months [5]. In the shallow, subtropical embayment of Shark Bay, an estimated 36% of seagrass meadows were damaged [2]. Wide-ranging effects associated with the heatwave were documented across lower trophic levels, while those on large vertebrates have remained more elusive. We investigated the vital rates

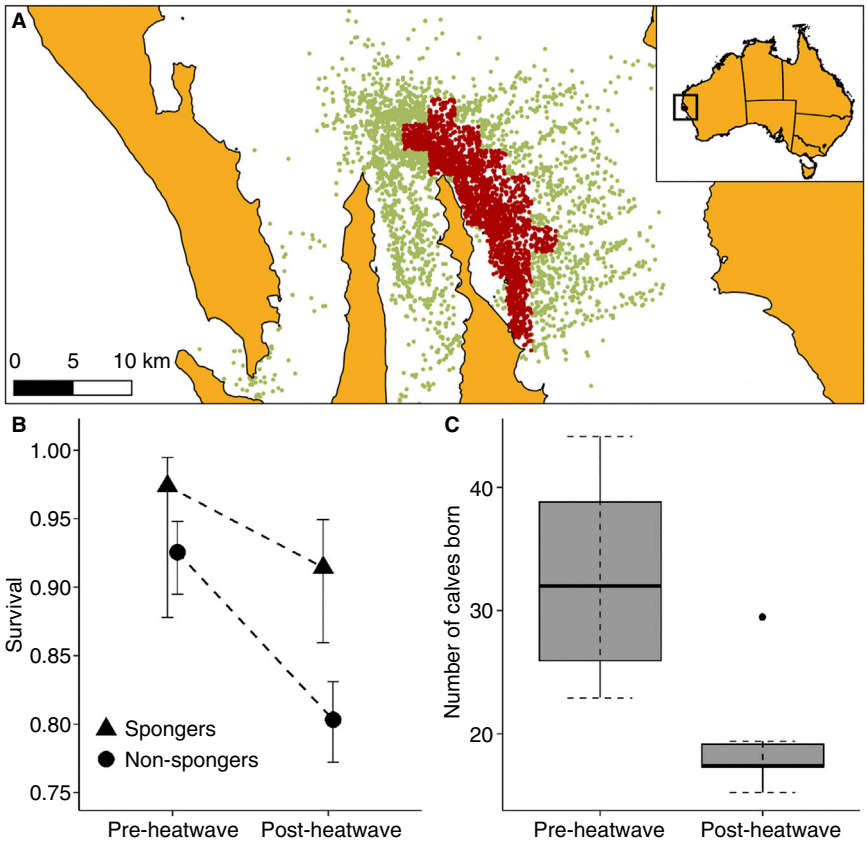

Figure 1. Dolphin vital rates in western Shark Bay before and after the 2011 marine heatwave. (A) The study area in the western gulf of Shark Bay, Western Australia, encompassing approximately 1,500 km². Over 5,000 dolphin group encounters have been documented between 2007 and 2017 (all points). To account for unequal survey effort in each field season, the study area was overlaid with a grid of 2 x 2 km cells. Only encounters within grid cells covered in all seasons ('core' study area) were considered for capture–recapture analyses (red points). (B) While both spongers and non-spongers experienced declines in survival from pre- to post-heatwave, spongers were less affected than non-spongers (5.9% decline versus 12.2% decline, respectively). (C) The number of calves detected (controlled for number of known mothers observed each field season) was significantly higher pre- compared to post-heatwave, suggesting that female dolphin reproductive success was also negatively impacted by habitat degradation and prey species loss following the heatwave.

(survival and reproduction) of dolphins in Shark Bay's western gulf, using long-term demographic and behavioural data collected between 2007 and 2017 (Figure 1A; see also Supplemental Information).

We assessed changes in apparent survival of dolphins over time using capture–recapture analyses (Data S1). Using Akaike weights as approximate model probabilities, there was a considerable decrease in survival after the heatwave. We suggest this was due to the well-documented losses of prey during the heatwave [3]. We also found that, on average, survival remained lower in the seven years after the heatwave than before. Again, having support from Akaike weights, we interpret this as evidence that dolphin mortality was impacted over a protracted period, rather than a single peak following the temperature anomaly (which had much lower support by Akaike weights). As such, the catastrophic reduction in seagrass coverage [2], which shows little sign of recovery [6], appears to be responsible for preventing fish stock recovery, since established seagrass meadows represent important breeding grounds and refuge habitat for numerous species [7].

We also tested for differences in survival between dolphins occupying different habitats, and those that use marine sponges as foraging tools ('spongers') versus those that do not

('non-spongers') [4]. Interestingly, while there was an overall decline in dolphin survival post-heatwave, survival of spongers was not as adversely impacted as that of non-spongers (5.9% versus 12.2% declines in survival from pre- to post-heatwave, respectively; Figure 1B). Sponge use is restricted to deeper channel habitats with no seagrass cover. While both spongers and non-spongers use these channels for foraging, sponge use allows access to a foraging niche which dolphins without the tool-using know-how cannot access [4], implying that the spongers' foraging niche may have been less severely impacted by the heatwave (Figure 1B).

A significantly greater number of calves per female per year was detected pre-heatwave compared with post-heatwave (Poisson generalized linear model (GLM); $z = -2.579$; effect size = 31.6%; 95% C.I. = [7.5%, 55.7%]; $p < 0.010$; Figure 1C), while no significant differences were found when comparing reproductive success for females from different habitat types (Poisson GLM; $z = 1.468$; $p = 0.142$), or using different foraging techniques (Poisson GLM; $z = -0.673$; $p = 0.501$) (Data S1). This result suggests that the spongers' access to a different foraging niche may have led to less severe effects on survival, but not reproduction. Reduced reproductive success as a consequence of significant ecological changes is not surprising, since a decline in food availability is expected to affect the most vulnerable members of a population, such as the young and those with high nutritional demands, like pregnant or lactating females [8].

There are several plausible explanations for this reduced reproductive success. First, lower prey availability may have forced female dolphins to spend more time foraging, leading to reduced vigilance and, ultimately, greater shark predation on calves. Second, reduced food availability can lead to either increased rates of abortion during pregnancy or increased neonate mortality, when both the mother's and the offspring's nutritional needs cannot be sustained [8]. Both abortions and increased mortality of young calves would effectively appear as lower reproductive success in the demographic data. Third, suppressed ovulation or delayed sexual maturity may have occurred when females did not reach a certain threshold of body weight [9]. Indeed, along with juvenile survival, fertility is the vital rate most sensitive to changes in resource availability in marine mammals [10].

Western Australia's 2011 marine heatwave negatively impacted habitat-forming and lower-trophic level organisms [2,3,5], and our results suggest cascading effects through to a behaviourally flexible, top-order predator. Long-lived taxa, such as some birds, great apes and cetaceans, are likely to experience changing ecological conditions throughout their lifetimes and, hence, may display behavioural plasticity in adapting to such changes. However, our findings suggest that the ecological consequences of extreme weather events may be too sudden or disruptive for even highly adaptable, animals to respond, leading to negative impacts on population viability. Such impacts may persist if ecosystems fail to recover. Further, our study adds to research illustrating that extreme events can drive biodiversity patterns and cause shifts in community structure toward more depauperate states [5]. These findings raise concerns over the long-term viability of the dolphin population, given that marine heatwaves are occurring with greater frequency in association with anthropogenic climate change [1].

SUPPLEMENTAL INFORMATION

Supplemental information includes two figures, one data file, supplemental experimental procedures and supplemental references and can be found at https://doi.org/10.1016/j.cub.2019.02.047. A video abstract is available at https://doi.org/10.1016/j.cub.2019.02.047#mmc3.

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AUTHOR CONTRIBUTIONS

Concept, S.W., M.K., S.J.A. Analysis, S.W., R.R., W.H. Investigation, S.W., S.J.A., L.G. Writing – Original Draft, S.W., S.J.A., M.K. Writing – Review & Editing, S.W., S.J.A., M.K., R.R., W.H., L.G. Funding, M.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Oliver, E.C.J., Donat, M.G., Burrows, M.T., Moore, P.J., Smale, D.A., Alexander, L. V, Benthuysen, J.A., Feng, M., Sen Gupta, A., Hobday, A.J., *et al.* (2018). Longer and more frequent marine heatwaves over the past century. Nat. Commun. *9*, 1–12.
2. Arias-Ortiz, A., Serrano, O., Masqué, P., Lavery, P.S., Mueller, U., Kendrick, G.A., Rozaimi, M., Esteban, A., Fourqurean, J.W., Marbà, N., *et al.* (2018). A marine heatwave drives massive losses from the world's largest seagrass carbon stocks. Nat. Clim. Chang. *8*, 338–344.
3. Pearce, A., Lenanton, R., Jackson, G., Moore, J., Feng, M., and Gaughan, D. (2011). The "marine heat wave" off Western Australia during the summer of 2010/11. Fish. Res. Rep. *No. 222*, 40pp.
4. Krützen, M., Kreicker, S., Macleod, C.D., Learmonth, J., Kopps, A.M., Walsham, P., and Allen, S.J. (2014). Cultural transmission of tool use by Indo-Pacific bottlenose dolphins (*Tursiops* sp.) provides access to a novel foraging niche. Proc. R. Soc. B *281*, 20140374.
5. Wernberg, T., Smale, D.A., Tuya, F., Thomsen, M.S., Langlois, T.J., de Bettignies, T., Bennett, S., and Rousseaux, C.S. (2012). An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. Nat. Clim. Change *3*, 78–82.
6. Nowicki, R.J., Thomson, J.A., Burkholder, D.A., Fourqurean, J.W., and Heithaus, M.R. (2017). Predicting seagrass recovery times and their implications following an extreme climate event. Mar. Ecol. Prog. Ser. *567*, 79–93.
7. Heithaus, M.R. (2004). Fish communities of seagrass meadows and associated habitats in Shark Bay, Western Australia. Bull. Mar. Sci. *75*, 79–99.
8. Trites, A.W., and Donnelly, C.P. (2003). The decline of Steller sea lions *Eumetopias jubatus* in Alaska: A review of the nutritional stress hypothesis. Mamm. Rev. *33*, 3–28.
9. Boyd, I.L., Lockyer, C., and Marsh, H.D. (1999). Reproduction in marine mammals. In Biology of Marine Mammals, J.E. Reynolds and S.A. Rommel, eds. (Washington, D.C.: Smithsonian Institution Press), pp. 218–286.
10. King, S.L., Schick, R.S., Donovan, C., Booth, C.G., Burgman, M., Thomas, L., and Harwood, J. (2015). An interim framework for assessing the population consequences of disturbance. Methods Ecol. Evol. *6*, 1150–1158.

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Applications Note

Genome analysis

SONiCS: PCR stutter noise correction in genome-scale microsatellites

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Abstract

Motivation: Massively parallel capture of short tandem repeats (STRs, or microsatellites) provides a strategy for population genomic and demographic analyses at high resolution with or without a reference genome. However, the high Polymerase Chain Reaction (PCR) cycle numbers needed for target capture experiments create genotyping noise through polymerase slippage known as PCR stutter.

Results: We developed SONiCS—Stutter mONte Carlo Simulation—a solution for stutter correction based on dense forward simulations of PCR and capture experimental conditions. To test SONiCS, we genotyped a 2499-marker STR panel in 22 humpback dolphins (*Sousa sahulensis*) using target capture, and generated capillary-based genotypes to validate five of these markers. In these 110 comparisons, SONiCS showed a 99.1% accuracy rate and a 98.2% genotyping success rate, mis-calling a single allele in a marker with low sequence coverage and rejecting another as un-callable.

Availability and implementation: Source code and documentation for SONiCS is freely available at https://github.com/kzkedzierska/sonics. Raw read data used in experimental validation of SONiCS have been deposited in the Sequence Read Archive under accession number SRP135756.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

For species lacking reference genomes, reduced representation sequencing methods such as target capture (Gnirke *et al.*, 2009), RAD-seq (Baird *et al.*, 2008), and genotyping-by-sequencing (Elshire *et al.*, 2011) can yield efficient datasets suitable for a wide range of genomic applications. These methods are typically used to develop sets of single nucleotide polymorphisms (SNPs), but target capture of genome-wide short tandem repeats (STRs) has recently emerged as a strategy for generating massively parallel datasets with rapid rates of evolution (Kistler *et al.*, 2017). STR capture circumvents the workflow bottlenecks of traditional STR development and genotyping while enhancing resolution in population genomic contexts compared with strictly SNP-based approaches. Furthermore, analyses of linked and co-phased SNPs and STRs allows for control of homoplasy that is frequently observed at STR loci, which can be identical by state without being identical by descent due to their high mutability (Ellegren, 2004).

A key challenge for STR capture is polymerase chain reaction (PCR) stutter—the physical slippage of DNA polymerase on the template strand causing molecules with different number of repeats

of the motif sequence to be synthesized (Schlötterer and Tautz, 1992). Stutter is a well-known obstacle for traditional STR genotyping based on amplicon size, and it remains confounding when using genomic STR methods (Gymrek *et al.*, 2012; Kistler *et al.*, 2017). In target capture experiments, a library amplification step is used both before and after probe–library hybridization totaling 20 or more PCR cycles (SOM), increasing opportunities for stutter-based alleles to appear and propagate through successive PCR steps. To address this issue, we developed a method for fitting the best diploid STR genotype to a set of raw allele counts by comparing the results of dense forward simulations.

2 Materials and methods

The complete SONiCS method is described in SOM, and outlined in Figure 1. Briefly, the user provides a set of raw reads supporting an unknown genotype and a starting pool of simulated molecules is generated using two independent alleles selected from the set of all possible genotypes present. A set of reaction parameters including the efficiency of amplification, efficiency of capture and the probability of polymerase slippage modeled separately for insertions and deletions are then drawn from weak uniform priors based on experimental observations or user inputs. The complete PCR and capture process is then modeled in-silico under the chosen parameters, and we calculate the likelihood of observing the input dataset from the product of the simulated starting genotype. After running a large number of independent simulations, we compare the distributions of the log likelihoods between all possible pairs of genotype calls that could be made. We then call a genotype on basis of a Bonferroni-corrected Mann–Whitney U test *P*-value and the likelihood ratios between the best fitting and second best fitting genotype.

SONiCS accepts either a single genotype provided at the command line or a VCF file including ALLREADS, MOTIF and REF fields as produced by allelotype, a component of lobSTR (Gymrek *et al.*, 2012). SONiCS writes a summary genotype file including the Mann–Whitney U test results, likelihood ratios for best and third quartile lnL values between alternative genotypes and the number of trials conducted. Optionally, SONiCS can also report the verbose parameters and results of each individual simulation for under all tested genotypes. This functionality allows completely flexible interrogation of simulation results and alternative filtering schemes for genotype selection according to the specific needs of stringency and experimental design. In order to optimize performance, large parts of the code for SONiCS are written in Cython (Behnel *et al.*, 2011), a superset of the Python programming language designed to give C-like performance with code mostly written in Python. The software is capable of using multiple processors and uses the 'multiprocessing' package from Python. On a single processor, SONiCS calculates 6–9 genotypes per minute on average. The signal to noise ratio at an STR locus depends on the coverage at the locus, the extent of the stutter, the repeat motif, the distribution of biological alleles and several other factors. Based on sub-sampling at validated loci (Supplementary Fig. S5, Supplementary Methods), we recommend using SONiCS on loci with a minimal coverage of 45 reads as a conservative threshold.

To test the accuracy of SONiCS, we used BaitSTR (Kistler *et al.*, 2017) and targeted resequencing to generate a set of 2499 STRs in a set of 22 Australian humpback dolphins (Sousa sahulensis; target regions provided as Supplementary Dataset S1). We then used lobSTR (Gymrek *et al.*, 2012) to align reads and summarize raw read support for target STRs and SONiCS to calculate resulting STR genotypes. We used traditional PCR and capillary genotyping to analyze five of the captured STR loci as a truth-set for comparison with SONiCS results (Supplementary Table S1). Complete genotyping procedures are described in SOM. Tissue samples were originally

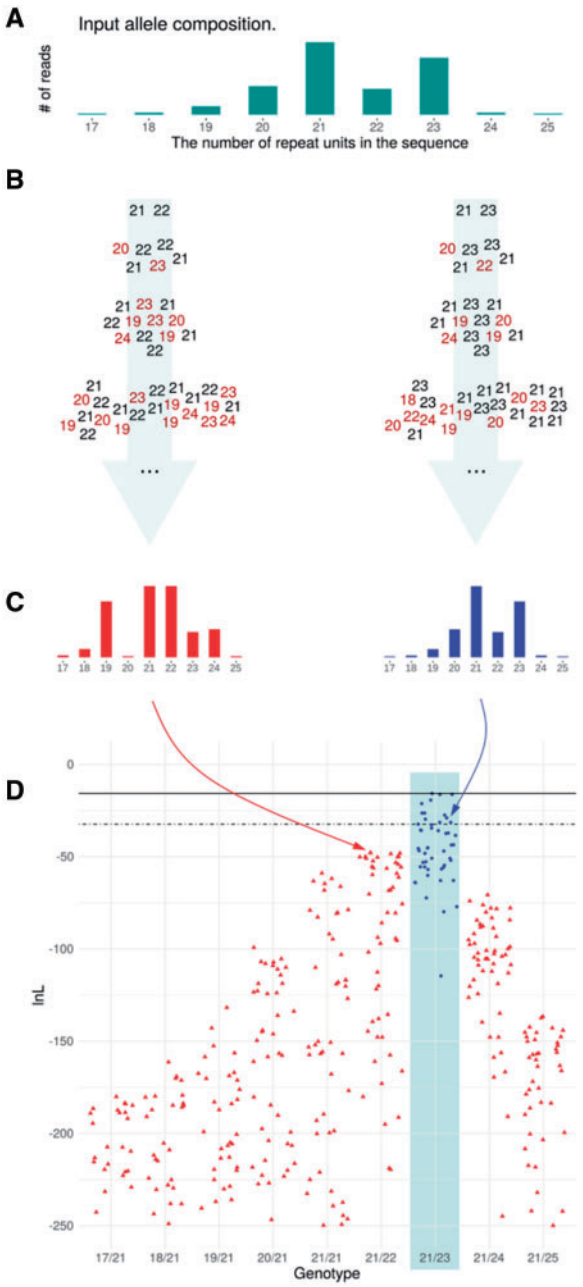

Fig. 1. Schematic of the SONiCS method. The user provides an empirical set of raw reads supporting alleles with different repeat counts (**A**). Several reactions that include PCR and the capture step are modeled *in silico* starting from various combination of input alleles, creating a post-PCR pool of molecules (**C**; new alleles in each cycle are shown in red). The log-likelihood (lnL) of sampling the starting read set from the output PCR pool is calculated as descried in SOM, along with the coefficient of determination and the percent identity for alternative filtering schemes. Next, the distributions of lnL values between genotypes (**D**) are compared using the Mann–Whitney U test and lnL ratios are calculated. A genotype is called if conditions of both tests are met. In this example, the input raw read support consists of alleles ranging from 17 to 25 repeat units, and SONiCS was able to determine the genotype of this particular locus as 21/23 after 500 simulations. Solid and dashed lines show the best single lnL and 75th percentile lnL under the best supported genotype

collected under permits from the Queensland Department of Environment and Heritage protection (WISP16457615) and combined permit of the Great Barrier Reef Marine Park Authority and Queensland Parks and Wildlife Service (G10/33405.1), with animal ethics committee approval from Southern Cross University.

3 Results

We recovered 95.2% of the complete STR panel across individuals through target capture—52 325 out of 54 978 possible STRs called—including all five markers overlapping capillary calls in all 22 samples. Across samples, a median 216 independent reads covered each marker, with between 13.0% and 18.9% of all reads per sample overlapping target STRs. After duplicate removal, this on-target proportion of reads equates to an effective 551-fold median enrichment of the target regions compared with whole genome shotgun sequencing data (range 393- to 807-fold). After SONiCS genotype calculation, we miscalled the capillary-validated genotype in only one instance—a 99.1% accuracy rate. SONiCS correctly rejected one additional genotype for failing the validation filters, yielding 108 genotypes for downstream analysis. The single miscalled locus was a heterozygous tetramer containing 10 and 11 repeats erroneously called as a homozygote with 11 repeats (Supplementary Tables S1 and S3). Coverage of the miscalled locus was in the 11th percentile of all markers (48x), and visual inspection confirms that the majority of reads ($n = 37$) supported the 11-repeat allele (Supplementary Table S2). Thus this specific locus is a difficult-to-resolve case where manual calling would suggest a noisy homozygote—consistent with SONiCS—and where allelic dropout and capture biases in the presence of low coverage may have confounded the underlying genotype.

4 Discussion

Existing methods for stutter correction in genomic sequence data primarily involve training a noise model on a large haploid subset of experimental data, such as a human Y-chromosome (Gymrek *et al.*, 2012). This approach provides an effective and replicable genotype likelihood rescaling method for whole-genome STR datasets, but is not typically applicable to STR capture datasets: First, species without a chromosome-level reference genome or lacking large haploid chromosomes—most plants, for example—cannot make use of this haploid training approach. Indeed a major advantage of the BaitSTR method (Kistler *et al.*, 2017) used here for marker development is that no genome assembly is required, and this benefit is incompatible with the lobSTR training framework. Second, even given a large haploid genomic region, reduced representation experiments would need to devote a large proportion of probe sets to training markers that may not be applicable to broader research priorities, and the training process itself might be confounded by the extreme depth and variation in coverage typical in capture experiments. Finally, STR makeup and base composition in flanking regions (e.g. GC content) have the potential to influence capture efficiency, PCR uptake and polymerase slippage during STR target capture. These variables would be difficult to unravel using a model-based noise correction method, but their influence is absorbed into the Monte Carlo approach using only weak uniform priors to constrain simulations.

For these reasons, we aimed to develop a stutter correction method that (i) can be used without a reference genome or an a priori noise model, and (ii) is robust to the idiosyncratic coverage variation and potential inter-locus biases of a target capture experiment. The statistical framework of SONiCS allows genotype selection in a strictly local context with only weak constraints on the parameters of STR fidelity and therefore confers both independence from any reference genome and tolerance to highly variable genomic representation in a target panel. Genotype calls from SONiCS matched our truth-set of capillary STRs in all but one of the cases, experimentally demonstrating that the SONiCS approach is effective for de-noising sequence-based STRs containing PCR stutter.

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References

Baird,N.A. *et al.* (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS One*, **3**, e3376.

Behnel,S. *et al.* (2011) Cython: the best of both worlds. *Comput. Sci. Eng.*, **13**, 31–39.

Ellegren,H. (2004) Microsatellites: simple sequences with complex evolution. *Nat. Rev. Genet.*, **5**, 435–445.

Elshire,R.J. *et al.* (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, **6**, e19379.

Gnirke,A. *et al.* (2009) Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nat. Biotech.*, **27**, 182–189.

Gymrek,M. *et al.* (2012) lobSTR: a short tandem repeat profiler for personal genomes. *Genome Res.*, **22**, 1154–1162.

Kistler,L. *et al.* (2017) A massively parallel strategy for STR marker development, capture, and genotyping. *Nucleic Acids Res.*, **45**, e142.

Schlötterer,C. and Tautz,D. (1992) Slippage synthesis of simple sequence DNA. *Nucleic Acids Res.*, **20**, 211–215.

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Genetic isolation between coastal and fishery-impacted, offshore bottlenose dolphin (*Tursiops* spp.) populations

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Abstract

The identification of species and population boundaries is important in both evolutionary and conservation biology. In recent years, new population genetic and computational methods for estimating population parameters and testing hypotheses in a quantitative manner have emerged. Using a Bayesian framework and a quantitative model-testing approach, we evaluated the species status and genetic connectedness of bottlenose dolphin (*Tursiops* spp.) populations off remote northwestern Australia, with a focus on pelagic 'offshore' dolphins subject to incidental capture in a trawl fishery. We analysed 71 dolphin samples from three sites beyond the 50 m depth contour (the inshore boundary of the fishery) and up to 170 km offshore, including incidentally caught and free-ranging individuals associating with trawl vessels, and 273 dolphins sampled at 12 coastal sites inshore of the 50 m depth contour and within 10 km of the coast. Results from 19 nuclear microsatellite markers showed significant population structure between dolphins from within the fishery and coastal sites, but also among dolphins from coastal sites, identifying three coastal populations. Moreover, we found no current or historic gene flow into the offshore population in the region of the fishery, indicating a complete lack of recruitment from coastal sites. Mitochondrial DNA corroborated our findings of genetic isolation between dolphins from the offshore population and coastal sites. Most offshore individuals formed a monophyletic clade with common bottlenose dolphins (*T. truncatus*), while all 273 individuals sampled coastally formed a well-supported clade of Indo-Pacific bottlenose dolphins (*T. aduncus*). By including a quantitative modelling approach, our study explicitly took evolutionary processes into account for informing the conservation and management of protected species. As such, it may serve as a template for other, similarly inaccessible study populations.

Keywords: bycatch, delphinids, gene flow, migration, population structure

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Introduction

Estimating population parameters such as effective population size, migration rate and its directionality, as well as the degree of admixture, are important in evolutionary biology. Whether individuals form part of a

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single, randomly mating population or are members of different populations with varying levels of genetic isolation also has important bearings on conservation and management (Waples & Gaggiotti 2006; Frankham *et al.* 2010). Genetic data are frequently employed to determine whether, and to what extent, samples collected at different locations are part of the same population or whether they are genetically differentiated, because information on geographic separation is not usually sufficient to determine the degree of isolation (Beerli & Palczewski 2010).

Genetic differentiation among populations may be observed in cases where there has been long-term separation with low recurrent gene flow, or recent divergence with no ongoing gene flow (Nielsen & Wakeley 2001; Palsbøll *et al.* 2004). Discriminating between these two scenarios has important ramifications for conservation, as isolated populations impacted by anthropogenic stressors may require different management strategies from those that experience homogenizing effects due to gene flow (Hoelzel *et al.* 1998b; Bilgmann *et al.* 2014).

Currently, there is no general framework outlining the levels at which populations are demographically independent (Waples & Gaggiotti 2006). In migration–drift equilibrium situations, assuming selective neutrality, genetic differentiation between populations is negatively correlated with the number of migrants per generation between them. Previous approaches inferred the number of migrants between populations based on the degree of genetic divergence between populations, such as Wright's F_{ST} (Wright 1931), based on a symmetric island model. However, it has been shown that these approaches are problematic, particularly as the mathematical model underlying the transformation of F_{ST} into the number of migrants per generation makes numerous assumptions, which are biologically unrealistic (e.g. Whitlock & McCauley 1999). More recently, individual-based methods have been developed that allow individuals to be assigned to populations using matching probabilities (e.g. Pritchard *et al.* 2000; Corander *et al.* 2008). Yet, these approaches are not able to estimate important population parameters, such as the directionality and extent of migration, mutation or population size, which may account for the present population structure (Palsbøll *et al.* 2007). Such information is important for assessing the impact of human activity on wildlife, but difficult to obtain in the marine environment.

Most cetacean species are impacted by human activities in at least some parts of their geographic range (Whitehead *et al.* 2000; Read *et al.* 2006). The incidental capture, or bycatch, of cetaceans in fisheries is a persistent threat to many populations (Halpern *et al.* 2007; Reeves *et al.* 2013), although knowledge of population structure and connectedness is currently lacking for many species. Gill netting, purse seining and trawling operations result in the greatest proportions of fishery-related mortalities (Read *et al.* 2006; Slooten 2013). In Australian waters, dolphins interact with prawn and fish trawling operations wherever they occur (e.g. Chilvers & Corkeron 2001; Svane 2005). Off the remote northwestern Australian coastline, dolphins regularly interact with the Pilbara Fish Trawl Interim Managed Fishery (Pilbara Trawl Fishery or 'PTF' hereafter, Jaiteh *et al.* 2013). Bycatch of a range of protected species (including dolphins, sawfish and turtles) was first highlighted in the PTF in 2002, with dolphin bycatch initially estimated at *c.* 50 individuals *per annum* (Stephenson & Chidlow 2003). An estimated minimum of 500 dolphins was caught in the 10 years from 2003 until 2012 (Allen *et al.* 2014).

The variable nature of cetacean–fisheries interactions requires species- and fishery-specific approaches to bycatch mitigation (Cox *et al.* 2004, 2007). Without any prior cetacean research having been conducted in the Pilbara region, the dolphin species interacting with the PTF was previously undetermined, but assumed to be the common bottlenose dolphin (*Tursiops truncatus*), based on a limited number of length measurements and photographs. Very little is known about common bottlenose dolphins in Australian waters (Ross 2006; Allen *et al.* 2012). Bottlenose dolphins are globally widespread in tropical and temperate waters, occurring in both coastal and pelagic populations (Rice 1998; Reeves *et al.* 2002). There are three putative *Tursiops* species in Australian waters: common bottlenose and Indo-Pacific bottlenose dolphins (*T. truncatus* and *T. aduncus*, respectively) have wide distributions (Woinarski *et al.* 2014) and the Burrunan dolphin (*T. australis*; Möller *et al.* 2008; Charlton-Robb *et al.* 2011; but see Committee on Taxonomy 2015), a proposed species that is restricted to a few southeastern Australian embayments. Common bottlenose dolphins are thought to occur further offshore and generally in deeper waters than Indo-Pacific bottlenose dolphins, which inhabit near-shore areas of much of the Australian coastline, including continental islands and reefs (Woinarski *et al.* 2014). Indo-Pacific bottlenose dolphins may mix with and/or be replaced by common bottlenose dolphins in some areas, and many communities of both these species interact with trawling operations around Australia (Allen *et al.* 2014; Woinarski *et al.* 2014). Although spinner dolphins (*Stenella longirostris* sp.) also occur in northwestern Australian waters and have been subject to bycatch in commercial fisheries (Ross 2006), they are morphologically and behaviourally distinguishable from the *Tursiops* Genus, and only the bottlenose dolphin phenotype

has been reported (by skippers, crew and fisheries observers) as bycatch in the PTF (Stephenson & Chidlow 2003; Allen *et al.* 2014).

While common bottlenose dolphins may occur in deeper waters than Indo-Pacific bottlenose dolphins around Australia, the 'offshore' dolphins interacting with the PTF do so between depths of *c.* 50 and 100 m (Jaiteh *et al.* 2013; Allen *et al.* 2014). This is not deep by oceanic standards, and coastal *T. aduncus* can be found in similar depths, especially when close to islands or where there is a steep gradient adjacent to the coast (Woinarski *et al.* 2014). Thus, one cannot assume *a priori* the absence of gene flow between the two groups of dolphins ('coastal' and 'offshore'), particularly given the high levels of hybridization among delphinids (e.g. Bérubé 2009; Schaurich *et al.* 2012; Brown *et al.* 2014).

Correct species identification is critical in wildlife management, because even closely related and morphologically similar species may possess variable behavioural and life history characteristics (Wade & Angliss 1997; Boness *et al.* 2002). Here, we used an extended population genetics toolbox to investigate the species status and population genetic structure of a number of bottlenose dolphin populations off northwestern Australia (Fig. 1), the first such study in this region. We collected small tissue biopsies from: incidentally captured and free-ranging dolphins interacting with the PTF; dolphins at multiple 'shallow' coastal sites inshore of the fishery and across northwestern Australia; and, dolphins in deeper waters off the North West Cape (Fig. 1). We aimed to determine whether dolphins interacting with the PTF showed greater genetic affinities to the common bottlenose dolphin (*T. truncatus*), the Indo-Pacific bottlenose dolphin (*T. aduncus*), or other closely related delphinid taxa. Furthermore, in addition to the traditionally used combination of basic genetic summary statistics and population structure analysis, we included explicitly model-based, coalescence analyses of genetic connectedness among dolphin populations across the region. In particular, we aimed to elucidate whether there was recruitment into the PTF-associated population(s) from nearby coastal sampling sites.

Materials and methods

Sample collection and fishery characteristics

Biopsy sampling efforts were focussed at 15 sites around northwestern Australia (Fig. 1). The Pilbara Trawl Fishery is bound by longitudes of 116°E to the west and 120°E to the east, and by an approximation of the 50 m depth contour inshore and the 100 m depth contour offshore (Fig. 1). Four management areas are open to trawl fishing, representing an area of *c.* 23 000 km^2. Three vessels operated in the PTF at the time of the study and they completed between *c.* 7300 and 10 300 h of trawling *per annum* from 2010 to 2012 (Fletcher & Santoro 2013).

All dolphin biopsy samples from within the fishery were collected between *c.* 50 and 170 km offshore, in water >50 m deep and over an east–west distance of *c.* 300 km. An eastern (Site 15) and a western (Site 14) cluster of samples were collected in the PTF (Fig. 1). Another three samples were collected in water *c.* 300 km to the southwest of the PTF: in deep (101 m) water offshore of the North West Cape (Site 13, 'NW Cape offshore', 114°E, Fig. 1). These three samples were included in this study to provide potential insight, albeit limited by the small sample size, into genetic connectedness of the PTF-associated dolphins to other 'offshore' populations.

Coastal biopsy sampling of bottlenose dolphins occurred at 12 sites in waters <50 m deep and within about 10 km of the coastline, extending from Useless Inlet (Site 1, 26.1°S, 113.3°E) in Shark Bay in the southwest to Cygnet Bay (Site 12, 16.5°S, 123.0°E) in King Sound in the northeast, spanning *c.* 2000 km of coastline (Fig. 1).

A total of 344 dolphin samples were collected between 2008 and 2013 (except those from Shark Bay, Sites 1–4, which were obtained between 1998 and 2013) and used for genetic analyses in this study. The subset of 68 samples of PTF-associated dolphins included three incidentally caught individuals and 65 free-ranging animals obtained during commercial fish trawling operations, on four trips to sea between 2008 and 2011. Biopsies from free-ranging dolphins were obtained using the PAXARMS remote biopsy system (Krützen *et al.* 2002) from a small (4.5 m) tender and a biopsy pole (Bilgmann *et al.* 2007) for sampling individual dolphins close to the bow or stern of trawl vessels (and a large research vessel for the three samples obtained in deeper waters offshore of the North West Cape). All 273 bottlenose dolphins sampled from the 12 coastal sites were collected from free-ranging dolphins using the PAXARMS remote biopsy system from small (5.5 m) research vessels.

Generation of genetic data

DNA was extracted from biopsy samples using the Qiagen Gentra tissue kit following the manufacturer's instructions. The extracted genomic DNA was resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and the concentration adjusted to 20 ng/μL. Sex determination was carried out by amplification of the sex specific ZFX and SRY loci using a multiplex PCR (Gilson *et al.* 1998).

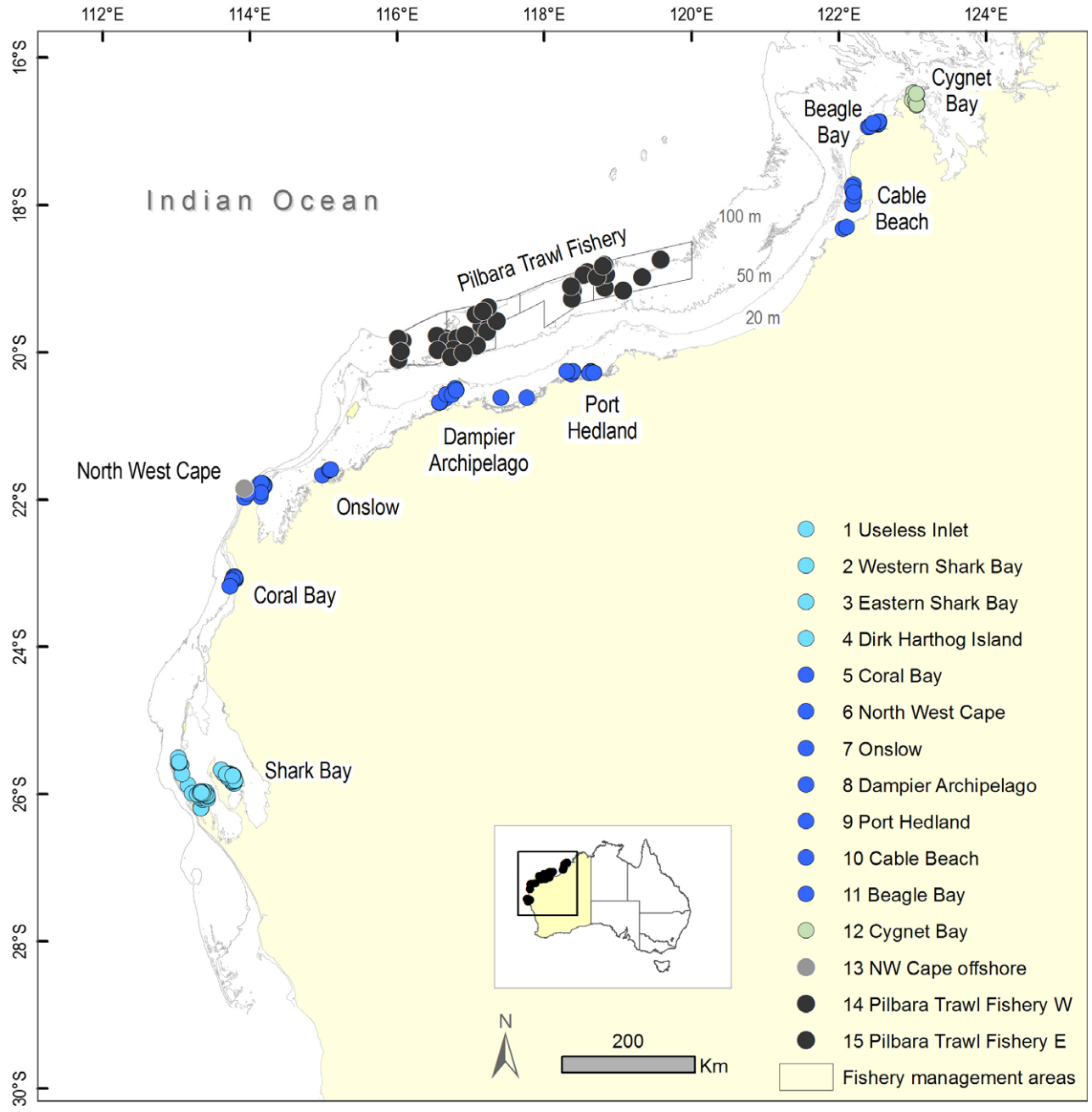

Fig. 1 Map of the sampling sites, northwestern Australia, showing the biopsy sample collection sites for: incidentally captured and free-ranging dolphins associating with the Pilbara Trawl Fishery (n = 68, black); Site 15, the 'PTF East' sampling site, was collected around 119°E, while 14, the 'PTF West' sampling site, was collected *c*. 160 km to the west, between *c*. 116°E and 117°E; dolphins in deep water off the North West Cape (n = 3, grey); and coastal dolphins (n = 273) from 12 sites (single circles may indicate multiple samples collected from some locations, light blue = Shark Bay sites, dark blue = other coastal sites, green = coastal Cygnet Bay). The boundaries of the PTF management areas and the 20-m-, 50-m- and 100-m- depth contours are also shown.

A 430-base pair part of the hypervariable region I of the mitochondrial control region (HVR-I) was amplified using primers dlp1.5 and dlp5 (Baker *et al*. 1993). PCR products were cleaned up using silica membrane spin columns (GeneElute™ by Sigma-Aldrich) and sequenced using the Cycle Sequencing Ready Reaction kit (BigDye Terminator v3.1 – Applied Biosystems), based on the protocol described in Bacher *et al*. (2010), using

sequencing primer dlp 1.5. SEQUENCING ANALYSIS v5.2 and BIOEDIT v7.0.5.3 were used to visually quality control, edit and align the sequences.

Nineteen microsatellite loci were amplified using two different multiplex PCR regimes: multiplex 1 – Tur4_98, Tur4_117, MK6, E12, Tur4_105, Tur4_108, Tur4_66, Tur4_111, Tur4_128 and multiplex 2 – KWM12, MK3, MK5, MK8, MK9, Tur4_142, Tur4_153, Tur4_162, Tur4_80, Tur4_132 (Hoelzel *et al.* 1998a; Krützen *et al.* 2001; Nater *et al.* 2009). PCRs contained 20 ng template DNA, 5 μL 2× Multiplex PCR Master Mix (Qiagen, containing HotStar*Taq* DNA Polymerase, dNTPs and 3 mM $MgCl_2$ final concentration), between 0.05 and 0.5 μM of each primer and molecular-grade water to a final volume of 10 μL. Diluted PCR products were denatured in 10 μL HiDi formamide containing 0.07 μL of GeneScanTM500LIZ size standard (Applied Biosystems). The length of the DNA fragments was determined by running the PCR products on an ABI 3730 DNA Sequencer (Applied Biosystems) and analysing the output files using GENEMAPPER v4.0. We independently amplified and scored 20 randomly selected individuals to estimate error rate for the microsatellite scoring. Our error rate was determined to be 0.0039 (three scoring differences in 760 alleles).

Population structure and gene flow

Population structure and genetic connectedness among sampling localities were inferred using both summary statistics and individual-based approaches based on microsatellite data. Genetic variation within sampling sites was estimated by calculating the number of alleles and effective alleles, observed (H_O), expected (H_E) and unbiased expected heterozygosity (UH_E) in GENALEX v6.5 (Peakall & Smouse 2012). Tests for departure from Hardy–Weinberg equilibrium (HWE) and the occurrence of linkage disequilibrium and null alleles were carried out for each sampling site in GENEPOP v4.2.1 (Rousset 2008), with Bonferroni-corrected significance levels (Rice 1989). Estimates of F_{ST} (Weir & Cockerham 1984) and Jost's D (Jost 2008) were calculated in GENEPOP and GENODIVE (Meirmans & Van Tienderen 2004), respectively.

The software package STRUCTURE v2.3.3 (Pritchard *et al.* 2000) was used to determine the genetic structure and number of genetic clusters in our data set. In particular, we were interested in the levels of genetic connectedness among the PTF-associated population(s) and the 12 coastal dolphin sampling localities. The STRUCTURE algorithm divides sampled individuals into a number of clusters (K) independent of locality information by minimizing deviations from Hardy–Weinberg and linkage equilibrium in each cluster. The software uses a Markov chain Monte Carlo (MCMC) procedure to estimate P(X|K), the posterior probability that the data fit the hypothesis of K clusters.

Three different STRUCTURE analyses were conducted. The length of the burn-in period was set to 10^5, followed by 10^6 MCMC steps. For each K (the maximum number of K for each analysis was the number of sampling locations for the respective analysis), the analysis was run 10 times. The first, global analysis involved all samples and used an admixture model with correlated allele frequencies and no prior information. For the two subsequent analyses, we chose the 'Locprior' model, which improves clustering when the signal is weak without spuriously inferring structure, if absent (Hubisz *et al.* 2009). The second analysis was carried out on PTF individuals only, while the third analysis incorporated only the 12 coastal populations. As the P(X|K) estimator has been shown to overestimate K, as it frequently plateaus at higher values than biologically meaningful estimates of K, we also calculated the ΔK statistic (Evanno *et al.* 2005). This provides a very conservative estimate of K only at the highest biological level and was performed using the software STRUCTUREHARVESTER (Earl & vonHoldt 2012).

In addition, a factorial correspondence analysis projecting all genotypes on the factor space, which is defined by the similarity of their allelic states, as implemented in GENETIX v4.05.2 (Belkhir *et al.* 2004), was used to visualize the degree of dissimilarity among sampling sites.

Migration patterns and gene flow among the PTF population and selected coastal populations were inferred based on two coalescence modelling approaches. The first approach was implemented in MIGRATE-N v3.6.4 (Beerli & Felsenstein 2001; Beerli 2006), which is based on an equilibrium island model to estimate genetic diversity of each defined population and all pairwise migration rates between these. This analysis was based solely on microsatellite data as the software does not implement a correction for differing inheritance modes, that is, mitochondrial DNA (mtDNA) vs nuclear DNA. In order to reduce the number of parameters in our models to arrive at a computationally and statistically tractable analysis, some relevant sampling sites were pooled into three populations (as identified in our STRUCTURE analysis, see Results): Pilbara Trawl Fishery (Sites 14–15), Shark Bay (Sites 1–4) and 'Other Coastal' populations (Sites 5–11; Cygnet Bay was excluded because our STRUCTURE and factorial correspondence analyses revealed at least some Cygnet Bay individuals to be genetically different). Four different models (Table 2) constraining the presence, directionality and amount of gene flow among the three pooled sampling sites were defined. Model 1 allowed full migration between all population pairs (full model).

One cannot define a model that sets migration among PTF and all other populations to nought because, under such circumstances, coalescence trees could not be calculated and general assumptions of the MIGRATE-N approach were violated. Therefore, model 2 allowed only very limited gene flow from and to PTF (≈ nought migration, but sufficient to match MIGRATE-N's needs with regard to coalescence trees). This effectively rendered the PTF population isolated from both Shark Bay and Other Coastal populations, while it allowed full migration between Shark Bay and Other Coastal populations (low migration PTF model). In model 3, gene flow from the PTF population into the Shark Bay and Other Coastal was allowed, but not vice versa. In model 4, gene flow from Shark Bay and Other Coastal populations to the PTF was allowed, but not vice versa. Convergence was achieved by running each model for more than 80 000 CPU hours, parallelized over 240 CPUs. We used 50 independent, replicate runs, each with its own burn-in and heating scheme, to later join the results (c.f., Hartmann *et al.* 2013). We regard the emergence of clear, unimodal posterior distributions across all these replicates as a strong indicator of convergence.

The run parameters for MIGRATE-N were as follows: for Θ (population size parameter, scaled to mutation rate) and *M* (migration rate parameter), a uniform prior was used. The prior range for Θ was set to 0–10 (mean 5; Δ 1; 20 000 bins) and for *M* 0–100 (mean 50; Δ 10; 20 000 bins). Mutation rates of loci were allowed to vary. Five hundred coalescent samples were recorded per replicate, one every 100 iterations, thus sampling 25 000 (50 × 500) parameter values from chains comprising a total of 2 500 000 iterations. A static heating scheme (4 chains with temperatures 1 000 000; 3; 1.5; 1) and a burn-in of 200 000 steps were applied to each replicate. Model comparisons were carried out using marginal likelihoods calculated using the thermodynamic integration ('Bezier') in MIGRATE-N (Beerli & Palczewski 2010). The estimated mutation-scaled migration parameter *M* was translated into the effective number of immigrants per generation (*Nm*), as detailed elsewhere (Jonker *et al.* 2013; Kraus *et al.* 2013).

The second coalescence approach to assess migration patterns and gene flow between the PTF population and selected coastal populations was implemented in IMA2 (Nielsen & Wakeley 2001; Hey & Nielsen 2007; Hey 2010). This approach is based on an isolation-with-migration (IM) model (allows for lack of gene flow, as opposed to MIGRATE-N) and uses Metropolis-coupled Markov chains to approximate posterior distributions of population size, gene flow and divergence time. Similar to our MIGRATE-N analysis, sampling sites were pooled into three populations (as identified in our STRUCTURE analysis, see Results): Pilbara Trawl Fishery (PTF, Sites 14–15), Shark Bay (Sites 1–4) and Other Coastal (Sites 5–11, i.e. Cygnet Bay excluded). In contrast to the MIGRATE-N analysis, however, we also included mtDNA data, because the software has a built-in ability to weigh across different inheritance modes. As IMA2 is slow for large multilocus data sets, we randomly selected 30 individuals from each of the three populations.

For the IMA2 analysis, we used uniform priors for divergence times and population sizes. For migration rates, exponential priors may be more informative when actual rates of gene flow are very low or nought (Runemark *et al.* 2012). However, among our three populations, gene flow might be substantial among the Other Coastal and Shark Bay populations. Thus, we also used uniform priors for migration rates. Mutation rates were set to 4.8×10^{-8} (range 3.1×10^{-8} to 6.9×10^{-8}; Oremus *et al.* 2007) mutations/year for mtDNA and 1.5×10^{-5} (Brohede & Ellegren 1999) for all microsatellite loci. Upper limits for divergence time were set to $t = 30$, population size $\Theta = 150$ and migration rate $M = 50$. The latter value appears high, but several initial runs (burn-in period of 20 000 and run length of 100 000) had shown that the parameter estimate of *M* between the Other Coastal and Shark Bay populations was very high. We carried out several independent runs. In each run, to ensure adequate mixing of the Markov chain, we used Metropolis coupling of 60 independent heated chains (Geyer 1992). Burn-in took place until stationarity was reached by assessing burn-trend plots for each run. The most heated chain had a heating factor of 0.9, with other chains having heating values between 1 and 0.9. As suggested by Hey (2010), stationarity for each run was evaluated by assessing autocorrelations of splitting time terms, the absence of trends in splitting time trend plots and by the degree of similarity between parameter estimates from genealogies generated during the first and the second half of the run.

To obtain estimates of magnitude and direction of contemporary gene flow between pairs of pooled populations, we used the software BAYESASS, v3.0.3 (Wilson & Rannala 2003). This approach uses an MCMC algorithm to estimate the posterior probability distribution of the proportion of migrants between pairs of populations without assuming genetic equilibrium. We used the same three population classifications as for the MIGRATE-N and IMA2 analyses, plus a fourth (Cygnet Bay, as BAYESASS accommodates for a larger number of populations with a moderate number of markers), and conducted five independent runs for 10 000 000 generations, while discarding the first 1 000 000 generations as burn-in. Mixing parameters for the five runs were $m = 0.3$, $a = 0.5$ and $f = 0.5$.

Finally, to learn more about coastal dolphin population structure, we conducted an isolation-by-distance analysis (Wright 1943) for all coastal populations, that is Shark Bay (Sites 1–4) and Other Coastal populations (Sites 5–12, i.e. including Cygnet Bay), based on our microsatellite data. Geographic distances between each sampling site were measured in the most direct line through the water using ArcGIS, v. 9.2 (ESRI), where the centroids for each population were estimated by including each sample taken at a particular site. We tested for a decrease in genetic similarity (based on F_{ST}) with increasing geographic distance, using a Mantel test implemented in IBDWS, v.3.23 (Jensen *et al.* 2005). Significance was evaluated by 10 000 randomizations.

Phylogenetic analyses

The mtDNA sequence alignment was trimmed to the shortest sequence, and part of the 5′ tRNA sequence was removed, resulting in a 399-bp fragment. Identical haplotypes were collapsed using Dambe v5.0.72 (Xia & Xie 2001). We used a general time-reversible model with gamma-distributed rate variation across sites and a proportion of invariable sites, as implemented in MrBayes v3.2, thereby sampling across the substitution model space in the Bayesian MCMC analysis itself (Huelsenbeck *et al.* 2004). Parameters for the MrBayes run were four chains running for 10 000 000 generations, with a sampling frequency of 1000 and a burn-in of 2500 data points. Consensus trees were displayed and printed using FigTree v1.1.2 (http://tree.bio.ed.ac.uk/).

To assess phylogenetic affiliations of the PTF-associated bottlenose dolphins with other delphinids, previously published HVR-I sequences from the following species and regions were included in the analysis: common (*T. truncatus*) and Indo-Pacific (*T. aduncus*) bottlenose dolphins, principally from Chinese and Indonesian waters (Wang *et al.* 1999), as well as the recently delineated Burrunan dolphin from Victoria, Australia (*T. australis*; Charlton-Robb *et al.* 2011) and Fraser's dolphins (*Lagenodelphis hosei*; Caballero *et al.* 2008; Table S1, Supporting information). We also included *T. aduncus* samples from coastal southeastern Australia (Möller & Beheregaray 2001; Möller *et al.* 2008; Wiszniewski *et al.* 2010). The tree was rooted with an Atlantic white-sided dolphin (*Lagenorhynchus acutus*; Cipriano 1997) sequence as an out-group (Table S1, Supporting information).

Results

Within each sampling site, all 19 microsatellite loci were in Hardy–Weinberg equilibrium. No significant linkage disequilibrium or consistent occurrence of null alleles in markers across all populations were observed. Allelic diversity and heterozygosity values were generally higher for the PTF samples compared with coastal sampling sites (Table S2, Supporting information). The pairwise F_{ST} values obtained from microsatellite data were small (generally <0.06), but significant among almost all sampling sites (Table 1). The highest values (generally >0.20) were observed for all pairwise comparisons between offshore and coastal sampling sites (Table 1). This suggests a longer period of isolation between offshore and coastal populations than among different coastal sampling sites. Pairwise values for Jost's *D* were generally larger than F_{ST} values. In particular, pairwise comparisons between PTF and coastal populations were on average 2–3 times larger for Jost's D than for F_{ST}, suggesting that F_{ST} underestimates divergence (Whitlock 2011).

For the global data set containing all samples, the Evanno method identified that $K = 2$ clusters was the most likely scenario. The Structure analysis illustrated a clear pattern of genetic differentiation between the offshore (both the PTF and NW Cape offshore) and all coastal sampling sites (Fig. 2a). For higher *K* values for the global data set, visual inspection revealed four clusters: (i) the four Shark Bay coastal sites, (ii) all coastal sites from Coral Bay to Beagle Bay, (iii) coastal Cygnet Bay and (iv) the NW Cape offshore and PTF (Fig. 2a).

When only PTF samples were considered, $K = 1$ had the highest probability, suggesting no genetic substructuring within the PTF. There was also no indication of any admixed individuals within the PTF, which could have been conceivable given the occurrence of Fraser's dolphin haplotypes (see below) in the PTF data set. When only coastal samples were considered (Fig. 2b), Shark Bay sites formed a distinct cluster from all other coastal sites, which was also supported by the Evanno method ($\Delta K = 2$). At $K = 3$ and higher, samples from Cygnet Bay became distinct, but the remaining coastal populations formed one cluster.

The factorial correspondence analysis based on 19 microsatellite loci (Fig. 3) strongly supported the Structure results. Samples from the PTF formed a single distinct cluster compared with all other samples, including NW Cape offshore. Among the coastal sites, the four Shark Bay sites in the southwest were clearly distinct from other sites across the northwest, while Cygnet Bay was distinct in the northeast. All other coastal sites could not be distinguished from each other (Fig. 3). An isolation-by-distance analysis on only coastal samples revealed a highly significant correlation ($r = 0.48$, $P < 0.01$) among all individual coastal sites (Fig. 4).

Based on the Structure results, we pooled most sampling localities into three 'populations' to analyse

Table 1 Pairwise F_{ST} (above) and Jost's D (below the diagonal) values between sampling sites

Sampling Site	Useless Inlet	Western Shark Bay	Eastern Shark Bay	Dirk Hartog Island	Coral Bay	North West Cape	Onslow	Dampier Archipelago	Port Hedland	Cable Beach	Beagle Bay	Cygnet Bay	NW Cape offshore	PTF West	PTF East
1 Useless Inlet		0.006	**0.031**	**0.038**	**0.059**	**0.042**	**0.025**	**0.041**	**0.033**	**0.060**	**0.046**	**0.065**	**0.251**	**0.279**	**0.263**
2 Western Shark Bay	0.025		**0.028**	**0.018**	**0.045**	**0.032**	**0.018**	**0.043**	**0.039**	**0.060**	**0.047**	**0.059**	**0.234**	**0.272**	**0.254**
3 Eastern Shark Bay	0.106	0.003		**0.036**	**0.054**	**0.066**	**0.044**	**0.053**	**0.039**	**0.060**	**0.058**	**0.082**	**0.259**	**0.279**	**0.263**
4 Dirk Hartog Island	0.225	0.041	0.004		**0.047**	**0.052**	**0.033**	**0.042**	**0.038**	**0.067**	**0.036**	**0.062**	**0.230**	**0.263**	**0.243**
5 Coral Bay	0.123	0.145	0.173	0.186		**0.015**	0.008	**0.020**	**0.025**	**0.028**	0.005	**0.051**	**0.233**	**0.264**	**0.244**
6 North West Cape	0.117	0.009	0.016	0.003	0.040		0.009	**0.020**	**0.032**	**0.040**	**0.022**	**0.067**	**0.285**	**0.291**	**0.276**
7 Onslow	−0.097	−0.126	−0.085	−0.004	0.076	−0.038		−0.006	0.017	0.006	0.009	**0.040**	**0.192**	**0.252**	**0.230**
8 Dampier Archipelago	0.104	0.001	−0.002	−0.004	0.063	−0.026	−0.097		**0.008**	0.010	**0.012**	**0.051**	**0.231**	**0.268**	**0.249**
9 Port Hedland	0.081	0.088	0.130	0.149	−0.059	0.023	0.001	0.032		**0.028**	**0.012**	**0.044**	**0.238**	**0.264**	**0.248**
10 Cable Beach	−0.016	0.101	0.107	0.253	0.178	0.175	−0.105	0.124	0.142		**0.036**	**0.057**	**0.199**	**0.253**	**0.233**
11 Beagle Bay	0.145	0.107	0.168	0.151	−0.051	0.021	0.093	0.062	−0.037	0.264		**0.047**	**0.230**	**0.256**	**0.233**
12 Cygnet Bay	0.250	0.214	0.225	0.188	−0.038	0.067	0.193	0.105	−0.001	0.324	−0.030		**0.199**	**0.247**	**0.231**
13 NW Cape offshore	0.491	0.774	0.685	0.873	0.706	0.835	0.480	0.721	0.685	0.235	0.892	0.861		**0.070**	**0.063**
14 PTF West	0.473	0.686	0.643	0.791	0.613	0.721	0.492	0.664	0.609	0.319	0.743	0.735	0.157		0.002
15 PTF East	0.418	0.626	0.574	0.721	0.563	0.661	0.396	0.589	0.551	0.246	0.701	0.692	0.092	−0.041	

Significant F_{ST} values (after Bonferroni correction, Rice 1989) are given in bold. The shaded area represents offshore–coastal population comparisons.

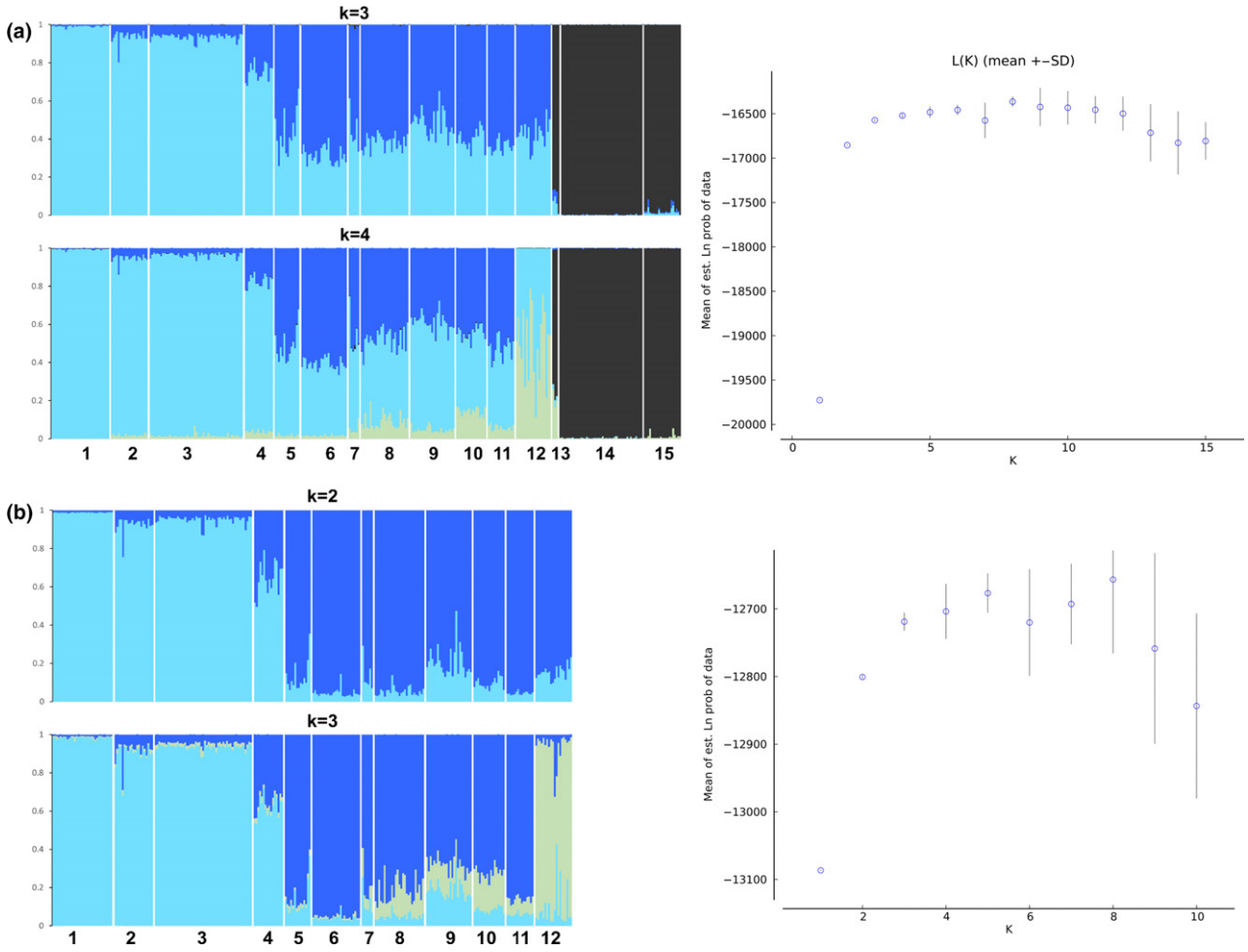

Fig. 2 Structure plots (each column representing assignment probability of an individual dolphin, with sampling sites separated by a white line) and log likelihoods for different number of clusters, *K*: (a) Full data set including all northwestern Australian samples (n = 344). (b) Coastal samples only (n = 273). The sampling site numbers correspond to their geographic location from the southwest to the northeast (coastal and then offshore) as in Fig. 1: 1 Useless Inlet; 2 Western Shark Bay; 3 Eastern Shark Bay; 4 Dirk Hartog Island; 5 Coral Bay; 6 North West Cape; 7 Onslow; 8 Dampier Archipelago; 9 Port Hedland; 10 Cable Beach; 11 Beagle Bay; 12 Cygnet Bay; 13 NW Cape offshore; 14 PTF West; 15 PTF East.

migration patterns among the combination of: (i) all four Shark Bay coastal sites ('Shark Bay'), (ii) all other coastal sites, other than Cygnet Bay ('Other Coastal') and (iii) PTF West and East into a single population ('PTF').

Our model comparisons showed a clear lack of migration into the PTF population from any of the coastal populations (Table 2). The model with the lowest support was that which allowed free migration among all populations (Table 2). Thus, our results suggest strongly that the PTF population is reproductively isolated from coastal populations, with no recruitment of dolphins into the PTF population from nearby coastal areas.

We based our parameter estimates of Θ (a mutation-scaled measure for population size) and *Nm* (the head count of effective migrants per generation) on the model that allowed estimation of Θ for all populations. As expected, Θ was highest for the pelagic PTF population (Θ = 6.37, 95% CI = 5.60–7.26). The coastal populations had smaller Θ values (Shark Bay Θ = 0.78, 95% CI 0.53–1.00; Other Coastal Θ = 2.90, 95% CI = 2.48–5.29). As there was no gene flow from the PTF to any of the coastal populations, we only report *Nm* estimates between the latter. The *Nm* estimate differed significantly from nought in both cases, with *Nm* values from Shark Bay to Other Coastal populations being higher than vice versa (SB → OC: *Nm* = 4.31, 95% *CI* = 3.70–7.89; OC → SB: *Nm* = 0.21, 95% CI = 0.14–0.26). Importantly, in all models, regardless of their level of support, the *Nm* parameter estimates concerning migration into the PTF population were always small and confidence

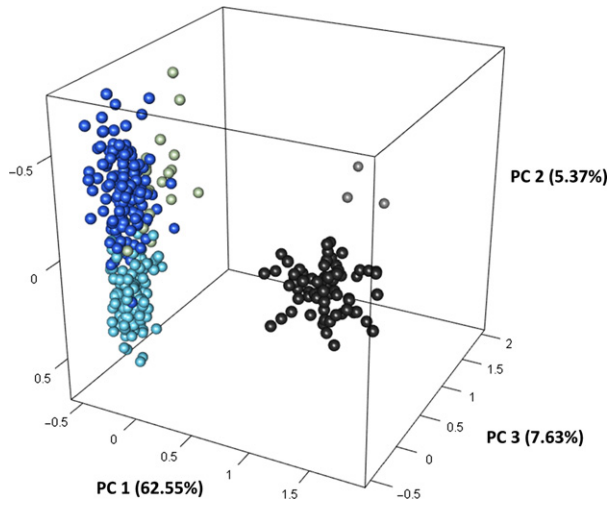

Fig. 3 Three-dimensional representation of a factorial correspondence analysis projecting all sampled individuals of northwestern Australian bottlenose (*Tursiops* spp.) dolphins on the factor space. The factor space is defined by the similarity of allelic states, in order to visualize the degree of dissimilarity among sampling sites. As per Figs 1 and 2: black/grey = individuals sampled in deeper (>50 m) waters in the PTF/NW Cape offshore; blue/green = individuals sampled in shallower (<50 m), coastal sites.

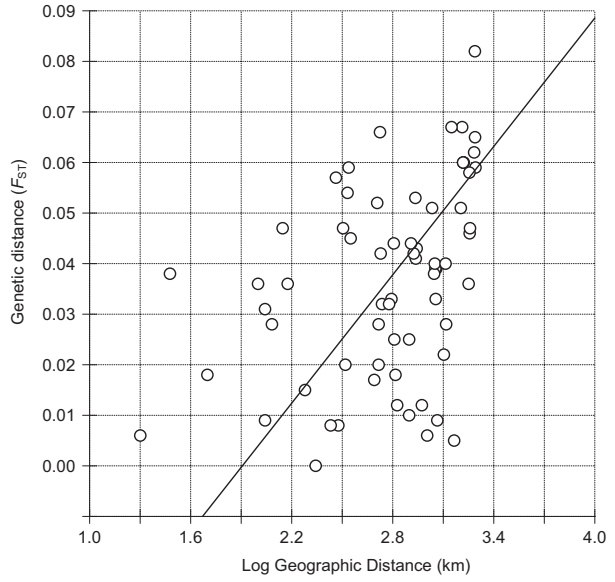

Fig. 4 Isolation-by-distance plot of correlation between genetic (F_{ST}) and geographic (km) distance for all coastal sampling locations (Sites 1–12), which is highly significant ($r = 0.482$, $P = 0.0011$).

intervals included 0, providing further evidence of the lack of recruitment of dolphins into the PTF population from nearby coastal areas. Result files for each model are available online as supplementary material.

Our IMa2 analyses corroborated those obtained by Migrate-n. Effective population size was largest for the PTF population (Fig. 5, Θ = 2.46, 95% CI = 1.57–3.5) and smaller for the coastal populations (Shark Bay Θ = 0.44, 95% CI 0.04–1.09; Other Coastal Θ = 1.65, 95% CI = 0.56–4.56). Migration rate parameters were only significant between Shark Bay and Other Coastal populations (log-likelihood ratio (LLR) = 5.45, $P < 0.01$) and vice versa (LLR = 2.87, $P < 0.05$, Fig. 5). The 95% confidence intervals of all migration parameters between the PTF and the two coastal populations included 0 and were not significant (Fig. 5), providing further evidence for the lack of gene flow between the PTF and all coastal populations.

The results from the Migrate-n and IMa2 analyses were corroborated by our findings based on BayesAss (Table 3). We could not detect any significant migration from the coastal populations into the PTF population and vice versa. In general, the proportion of detected migrants within each population (other than Cygnet Bay, which received about 28% of migrants from the Other Coastal population) was small, and the 95% confidence interval included 0 in almost every comparison. These findings suggest strongly that there is no, or at most only extremely low, migration between the PTF, and the Shark Bay and Other Coastal populations.

Table 2 Comparisons of four different migration models used in Migrate-n. For model comparisons, we pooled sampling sites into three populations (see Materials and methods)

Model	Populations and model parameters			Bezier approximation score (BAS)	ΔBAS
	SB	OC	PTF		
Full	***	***	***	−359 466	133 001
Low migration PTF	**c	**c	cc*	−237 198	10 733
PTF → SB/OC	***	***	00*	−226 465	0
SB/OC → PTF	**0	**0	***	−260 614	34 149

SB = Shark Bay (Sites 1–4), OC = Other Coastal (Sites 5–11), PTF = Pilbara Trawl Fishery (Sites 14–15). In each of the migration models, nine parameters, that is, migration rates between populations, were considered. Asterisks indicate that migration rates were estimated by Migrate-n. In some migration models, we set the migration rate among certain populations to nought (0), or allowed only a fixed, low (c) migration rate of 0.001 (see Materials and methods). Parameters 1–3 indicate migration rate into the SB populations from the SB, OC and PTF populations, parameters 4–6 indicate migration rate into the OC population from the SB, OC and PTF populations, and parameters 7–9 indicate migration rate into the PTF population from the SB, OC and PTF populations. Model scores are given by Bezier approximation and differences between models highlighted in column ΔBAS.

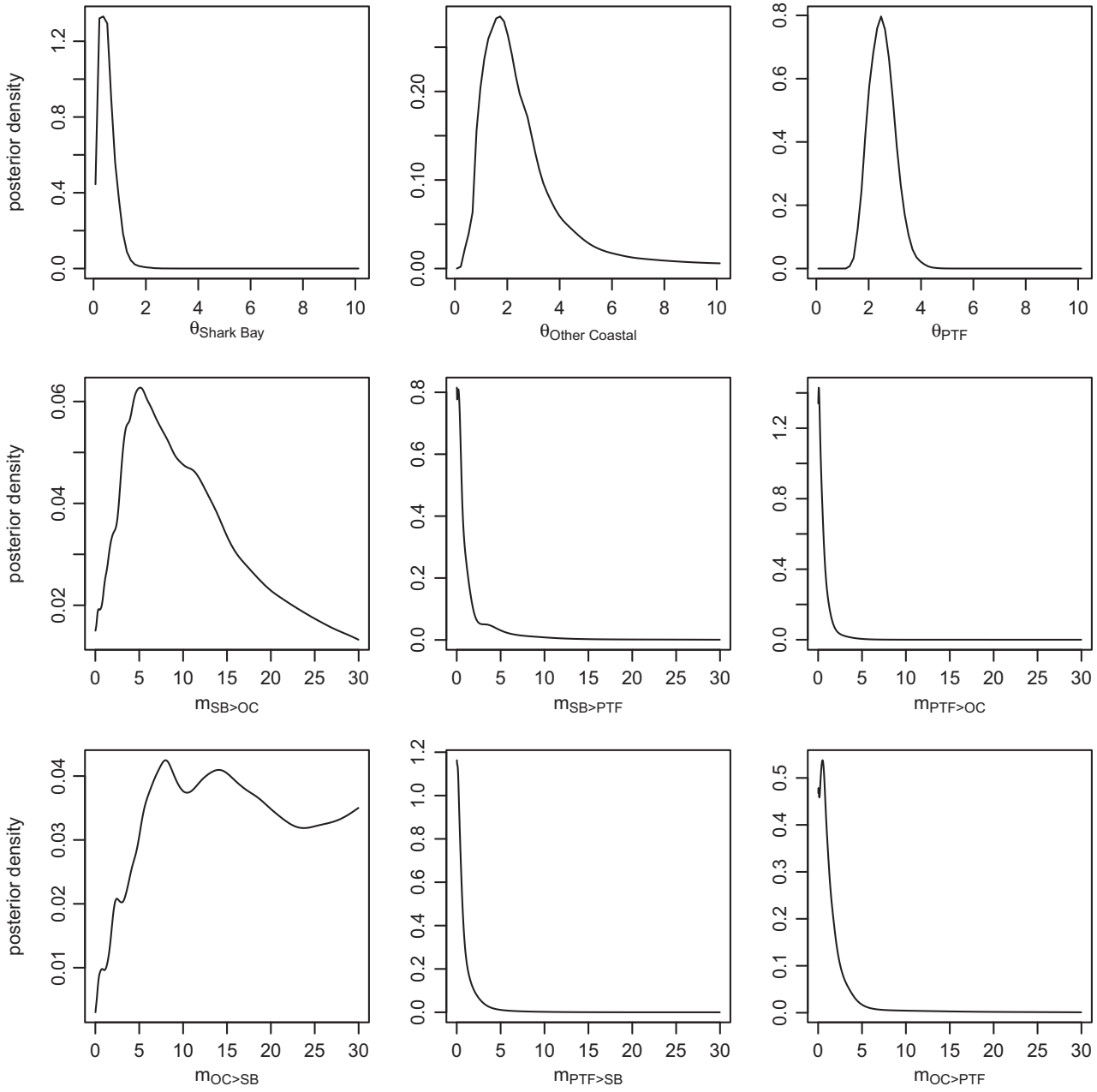

Fig. 5 Posterior density distributions for IMa2 simulations. Θ = relative effective population size, m = migration rate, PTF = Pilbara Trawl Fishery, SB = Shark Bay, OC = Other Coastal.

The phylogenetic analyses based on mtDNA revealed some unexpected patterns. We identified 17 unique haplotypes among all individuals collected from within the two sampling sites in the PTF (Sites 14 and 15, Fig. 1), as well as those collected in deep water offshore of the North West Cape (Site 13). These haplotypes formed a well-supported, monophyletic clade with the common bottlenose dolphin. Within this clade, however, clear resolution was lacking (Fig. 6). The haplotype of six individuals sampled within the fishery formed a well-supported monophyletic clade (posterior probability of 0.97) with Fraser's dolphin haplotypes (Fig. 6), an unexpected result that is discussed below. While at-sea differentiation among delphinids can be difficult, all observations and photographs taken during offshore field trips were of the common bottlenose dolphin phenotype. All of the bottlenose dolphins sampled in the coastal regions of northwestern Australia formed a highly supported monophyletic clade (posterior probability of 1.00) with other Indo-Pacific bottlenose dolphins (*T. aduncus*; Figs 1, 6).

Table 3 Mean posterior distribution values (95% CI) of fraction of individuals in population *i* that are migrants derived from population *j* (per generation) among four combined populations as determined by BAYESASS. 95% confidence intervals smaller than 0 and larger than 1 were rounded to the nearest integer

From/to	Shark Bay	Other Coastal	Cygnet Bay	PTF
Shark Bay	0.982 (0.963–1.000)	0.013 (0.000–0.031)	0.003 (0.000–0.008)	0.003 (0.000–0.008)
Other Coastal	0.034 (0.000–0.071)	0.961 (0.924–0.998)	0.003 (0.000–0.007)	0.003 (0.000–0.007)
Cygnet Bay	0.022 (0.000–0.059)	0.284 (0.235–0.334)	0.681 (0.654–0.707)	0.013 (0.000–0.037)
PTF	0.005 (0.000–0.014)	0.005 (0.000–0.014)	0.005 (0.000–0.014)	0.986 (0.971–1.000)

Discussion

Lack of contemporary and historic gene flow between fishery-impacted and coastal dolphins

All our analyses based on nuclear microsatellite data suggest strongly that the bottlenose dolphins sampled in the offshore, pelagic environment (*Tursiops truncatus*) are genetically isolated from those sampled coastally (*T. aduncus*). Both the STRUCTURE and factorial correspondence analyses revealed four clusters of individuals that were geographically separated (one offshore and three coastal 'populations'). Similarly strong patterns of segregation have been reported in other small cetaceans. For example, Perrin *et al.* (2011) used cranial osteological differentiation to support previous assertions, based on molecular data, for the existence of coastal and offshore forms of common bottlenose dolphins (*T. truncatus*) in Californian waters. Also, false killer whale (*Pseudorca crassidens*) populations sampled offshore in the central and eastern Pacific were recently differentiated from those that are resident and island-associated around the Hawai'ian Archipelago (Martien *et al.* 2014).

Our STRUCTURE and factorial correspondence analyses, however, did not reveal whether the genetic isolation between the PTF and coastal populations is due to historic cessation of gene flow (i.e. reproductive isolation followed by speciation), or recent divergence. All analyses pertaining to migration rates revealed an absence of gene flow from any coastal population into the PTF population and vice versa, strongly suggesting that the bottlenose dolphin population that is subject to incidental capture in the PTF is genetically isolated from all the adjacent, coastal dolphins and does not recruit from these coastal dolphin populations. Furthermore, we found no evidence of hybridization between the pelagic common bottlenose dolphins (*T. truncatus*) and the coastal Indo-Pacific bottlenose dolphins (*T. aduncus*). This finding is consistent with that for these two species in Chinese waters, which, despite some areas of overlap in distribution, were found to be reproductively isolated and did not share mtDNA haplotypes (Wang *et al.* 1999; Yang *et al.* 2005).

In our study, the pelagic common bottlenose dolphins showed less genetic substructuring than the coastal Indo-Pacific bottlenose dolphins sampled across a similar geographic distance. Furthermore, the mutation-scaled, effective population sizes (Θ) of common bottlenose dolphins were much larger than those of the Indo-Pacific bottlenose dolphin population in Shark Bay and the combined coastal populations. These results were to be expected, given the more complex coastal habitat, environmental and social barriers to gene flow, and limited dispersal (Krützen *et al.* 2004; Frère *et al.* 2010), as well as the propensity for coastal *Tursiops* of both species to adapt rapidly to local habitats (e.g. Hoelzel *et al.* 1998b; Sellas *et al.* 2005; Wiszniewski *et al.* 2010). Common bottlenose dolphins of open, pelagic environments are capable of long-distance movements: for example, Wells *et al.* (1999) documented travel distances of *c.* 2000 and >4000 km in < 50 days by two satellite-tracked individuals off the east coast of the United States. Furthermore, Quérouil *et al.* (2007) found no genetic differentiation among common bottlenose dolphins from the Azores, Madeira and other offshore areas of the northeast Atlantic, suggesting that they form a large, pelagic population. The lack of baseline data on Australian common bottlenose dolphins means it is not possible to assess whether the population in the PTF region is an isolated unit or forms part of a large, pelagic population (Ross 2006). The relatively large Θ supports the latter view, but our factorial correspondence analysis revealed some segregation between common bottlenose dolphins in the PTF and those of the North West Cape (Fig. 3), and photographic evidence shows that at least a proportion of the PTF-associated population display long-term fidelity to foraging around the trawlers (Allen 2015).

Pelagic, common bottlenose dolphins of northwestern Australia

Most dolphins associated with the PTF, as well as those sampled in deeper (>50 m) waters off the North West Cape, exhibited haplotypes that form a monophyletic clade with those previously published for common

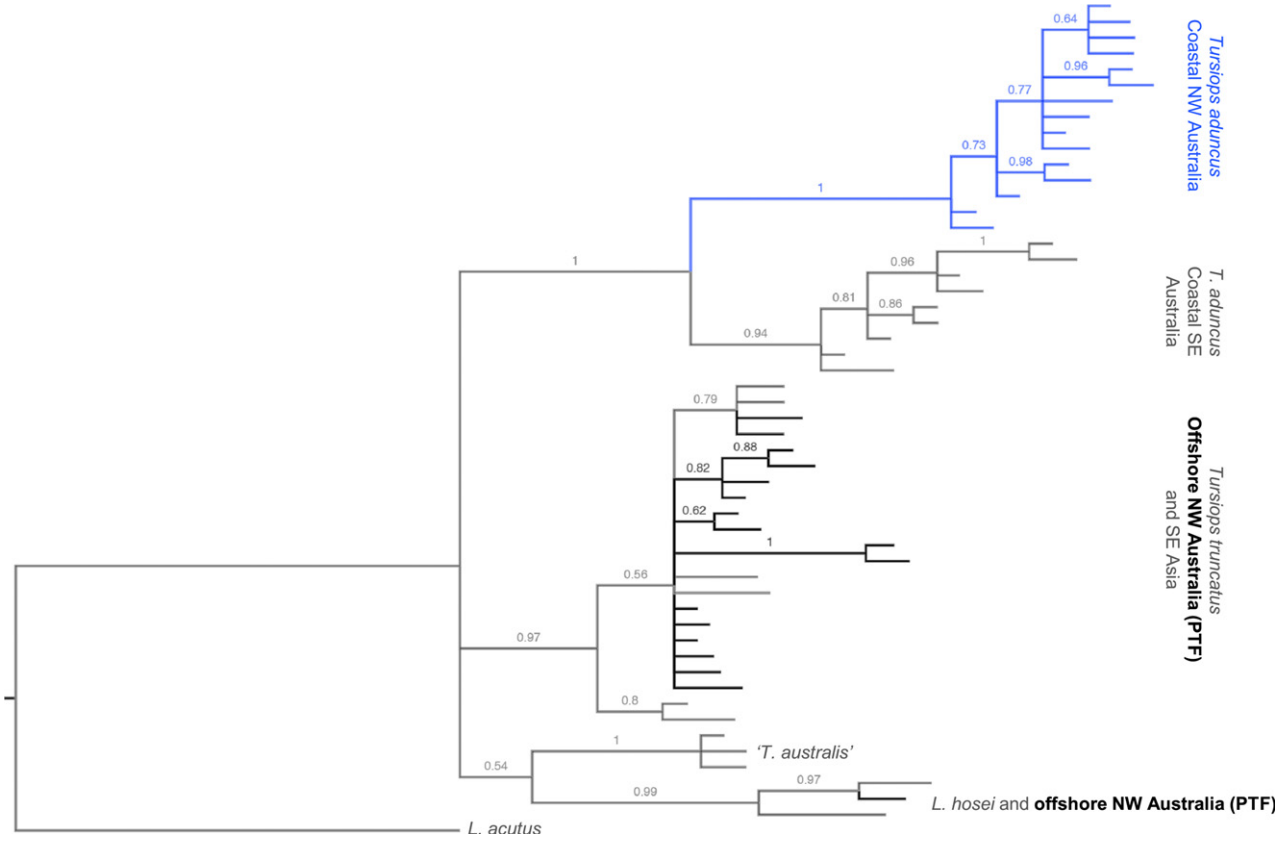

Fig. 6 Phylogenetic relationships of offshore northwestern Australian (Pilbara Trawl Fishery and North West Cape) dolphin mtDNA haplotypes and coastal northwestern Australian dolphin mtDNA haplotypes compared to relevant delphinids, based on an alignment of 399 base pairs of the hypervariable region I. Node labels are posterior probabilities. Taxa in black branches are PTF-associated samples from this study. Coastal samples from this study (blue) formed a reciprocally monophyletic clade to previously published *Tursiops aduncus* from coastal southeast Australia. Taxa in grey branches are those from other studies.

bottlenose dolphins (*T. truncatus*) from Chinese and Indonesian waters. Until the current study, the Chinese and Indonesian haplotypes were the only available reference samples for *T. truncatus* in this region, despite the fact that they are globally widespread in both coastal and pelagic populations (Rice 1998; Reeves *et al.* 2002).

Bottlenose dolphins are polytypic, with two species recognized based on both genetics and morphology: *T. truncatus* and *T. aduncus* (Rice 1998; Wang *et al.* 1999, 2000a,b), and a third species proposed recently: *T. australis* (Möller *et al.* 2008; Charlton-Robb *et al.* 2011). The number of species/subspecies in the complex, however, remains to be resolved (e.g. Natoli *et al.* 2004), with the Society for Marine Mammalogy currently recognizing only *T. truncatus* and *T. aduncus* (Committee on Taxonomy 2015). All three putative species are present in Australian waters, with *T. truncatus* generally thought to occur further offshore and in deeper waters than *T. aduncus* (Ross 2006), a pattern confirmed for northwestern Australia in this study.

The use of the mitochondrial control region for phylogenetic species identification has also proven effective in a range of other studies for closely related delphinids (e.g. Rosel *et al.* 1994; Möller & Beheregaray 2001; Ross *et al.* 2003; Beasley *et al.* 2005). This marker system also has limitations, however, and its usefulness for species identification depends on the evolutionary distinctiveness of the taxa in question. In studies attempting to elucidate the evolutionary relationships among the Delphininae, the use of a single mitochondrial gene has provided limited resolution, due to high levels of intraspecific variation and low interspecific differences (Kingston *et al.* 2009; Viricel & Rosel 2012). Kingston *et al.* (2009) found that data from amplified fragment length polymorphisms (AFLPs), representing many nuclear genes, gave better resolution. However, even the use of genomewide multilocus data sets such as this, and others (Xiong *et al.* 2009; McGowen 2011; Zhou *et al.* 2011), has not been able to resolve relationships unambiguously within the Delphininae, which is thought to have undergone a recent and rapid radiation

(Kingston *et al.* 2009; Vilstrup *et al.* 2011; Hassanin *et al.* 2012).

In our study, both *T. truncatus* and *T. aduncus* formed well-supported monophyletic clades, as has been documented elsewhere (e.g. Möller & Beheregaray 2001; Moura *et al.* 2013). Most individuals from within the PTF, and elsewhere offshore, fell within the *T. truncatus* clade, providing strong evidence that it is predominantly common bottlenose dolphins associating with the fishery. These results were corroborated by the lack of both historic and contemporary gene flow between the PTF-associated common and coastal Indo-Pacific bottlenose dolphins, identified from our microsatellite data and two independent approaches to estimate gene flow.

Unexpectedly, some offshore individuals exhibited a haplotype that shares a close affinity to Fraser's dolphin haplotypes. Fraser's dolphins occur primarily in waters deeper than 1000 m (Reeves *et al.* 2002). They are rarely found in shallow waters or near-shore environs, and field guides and texts report Fraser's dolphins in mixed-species assemblages only with false killer, melon-headed (*Peponocephala electra*) and sperm (*Physeter macrocephalus*) whales, as well as Risso's (*Grampus griseus*), pan-tropical spotted (*Stenella attenuata*) and striped (*S. coeruleoalba*) dolphins (Carwardine 1995; Reeves *et al.* 2002; Dixon 2008; Jefferson *et al.* 2008). Fraser's dolphins have not been observed in mixed assemblages with bottlenose dolphins, nor would they be expected in the relatively shallow waters (50–100 m deep) in which the PTF operates.

There are three plausible explanations for the occurrence of the Fraser's dolphin haplotypes among the PTF-associated dolphins. First, both *T. truncatus* and *L. hosei* may have been present in the groups of dolphins that were sampled. However, a careful re-examination of all photographs taken in the field revealed only the bottlenose dolphin phenotype, and the STRUCTURE analysis did not reveal any admixed individuals within the PTF. Second, incomplete lineage sorting may have led to the observed pattern. Under a neutral model of evolution, the stochastic lineage sorting leading to reciprocal monophyly proceeds more slowly in large or rapidly diverging populations. In many groups of species with large population size, such as the Delphinidae (Rice 1998; McGowen 2011), genomes will have mixed support for monophyly unless historical bottlenecks have accelerated coalescence. For instance, Kingston *et al.* (2009) used anonymous nuclear and mtDNA markers to elucidate the phylogenetic relationships among the Delphininae. In their analysis, *L. hosei* showed high affinity to *T. aduncus* for both marker systems, suggesting recent shared ancestry between *Tursiops* and *Lagenodelphis*. A third explanation for the occurrence of Fraser's dolphin haplotypes among the PTF-associated bottlenose dolphins is that introgression events have taken place, in which Fraser's dolphin mtDNA entered the population through hybridization.

Coastal, Indo-Pacific bottlenose dolphins of northwestern Australia

Indo-Pacific bottlenose dolphins (*T. aduncus*) inhabit near-shore areas of much of the Australian coastline (Ross 2006; Woinarski *et al.* 2014; this study). Occurring in the shallow, coastal waters of the western Pacific and Indian Oceans, Indo-Pacific bottlenose dolphins thereby occupy a niche otherwise filled by coastal ecotypes of common bottlenose dolphins in various other regions (e.g. the coastlines of New Zealand, the central and eastern Pacific Ocean, the western and eastern Atlantic Oceans and the Mediterranean Sea – Natoli *et al.* 2005; Tezanos-Pinto *et al.* 2009; Moura *et al.* 2013; Fruet *et al.* 2014).

Our study also revealed a strong isolation-by-distance pattern among coastal Indo-Pacific bottlenose dolphin (sub-) populations across northwestern Australia. Fine-scale genetic structuring over scales of just tens to hundreds of kilometres should be viewed as the rule rather than the exception in coastal Australian Indo-Pacific bottlenose dolphins (e.g. Wiszniewski *et al.* 2009; Ansmann *et al.* 2012; Kopps *et al.* 2014), as it should be in coastal common bottlenose dolphins globally (Fernández *et al.* 2011; Mirimin *et al.* 2011; Moura *et al.* 2013; Browning *et al.* 2014; Fruet *et al.* 2014; Louis *et al.* 2014). Here, however, we document the existence of a genetic cline among coastal locations over some hundreds of kilometres (Beagle Bay to Coral Bay, Fig. 1).

An exception to this was the marked genetic differentiation between the dolphins sampled at the two extreme northeast coastal sites (Cygnet Bay and Beagle Bay), located in close proximity to each other (<150 km apart). The dolphins from Beagle Bay, however, clustered closely with the rest of the coastal populations, distinct from Cygnet Bay. Similar differentiation was detected between Australian snubfin dolphin populations of Cygnet Bay and Roebuck Bay (to the south of Beagle Bay), *c.* 300 km apart (Brown *et al.* 2014). The reasons for this differentiation, which was detected at a smaller spatial scale than elsewhere in the study area, are unknown. The relatively narrow, deep-water entrance to Cygnet Bay, subject to immense tidal movements (*c.* 12 m on spring tides), may act as a natural barrier to dispersal. Additional sample collection to the east of Cygnet Bay, the incorporation of detailed habitat data (e.g. bathymetry, substrate type) and large-scale genomic data will better elucidate the patterns and potential drivers of genetic connectedness among

coastal populations of bottlenose dolphins across northwestern Australia.

Conclusions and recommendations

This study provides evidence that the common bottlenose dolphin (*T. truncatus*) is the predominant species associating with the Pilbara Trawl Fishery and that haplotype sharing or recruitment from adjacent, coastal populations (*T. aduncus*) does not occur. There appears to be no genetic substructuring within the PTF-associated population. Data on population size need to be acquired before the viability, or capacity to absorb and recover from, the anthropogenic impact of ongoing incidental catch, at an estimated minimum of *c.* 50 dolphins *per annum*, can be assessed (Allen *et al.* 2014). A more complete biopsy sample data set, from offshore *T. truncatus* populations adjacent to the PTF, needs to be accumulated to allow the quantification of the levels of gene flow with adjacent, pelagic populations. This might also allow: the detection of any changes in population size due to fishery-caused mortalities (c.f., Garza & Williamson 2001); the determination of whether closely related individuals are subject to incidental capture (c.f., Mendez *et al.* 2010), which can exacerbate the demographic impacts of bycatch in highly social species, such as delphinids (Wade *et al.* 2012); and, the definition of appropriate management units for pelagic dolphins across northern Australia (c.f., Bilgmann *et al.* 2014). Finally, underwater video footage collected inside trawl nets (Jaiteh *et al.* 2014), as well as photo-identification data from around trawlers (Allen 2015), suggests that a community of dolphins within the broader population may show fidelity to foraging around trawlers. Estimating the number of individuals interacting with the trawlers is also required to better assess the level of impact this putative community faces.

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References

Allen SJ (2015) Fishery-impacted bottlenose dolphins of northwestern Australia: Bycatch patterns, genetic status and abundance. Doctoral thesis. School of Veterinary and Life Sciences, Murdoch University, Australia. 144pp.

Allen SJ, Cagnazzi DDB, Hodgson AJ, Loneragan NR, Bejder L (2012) Tropical inshore dolphins of north-western Australia: unknown populations in a rapidly changing region. *Pacific Conservation Biology*, **18**, 56–63.

Allen SJ, Tyne J, Kobryn HT, Bejder L, Pollock KH, Loneragan NR (2014) Patterns of dolphin bycatch in a north-western Australian trawl fishery. *PLoS ONE*, **9**(4), e93178.

Ansmann IC, Parra GJ, Lanyon JM, Seddon JM (2012) Fine-scale genetic population structure in a mobile marine mammal: inshore bottlenose dolphins in Moreton Bay, Australia. *Molecular Ecology*, **21**, 4472–4485.

Bacher K, Allen S, Lindholm AK, Bejder L, Krützen M (2010) Genes or culture: are mitochondrial genes associated with tool use in bottlenose dolphins (*Tursiops* sp.)? *Behavior Genetics*, **40**, 706–714.

Baker CS, Perry A, Bannister J *et al.* (1993) Abundant mitochondrial DNA variation and worldwide population structure in humpback whales. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 8239–8243.

Beasley I, Robertson KM, Arnold P (2005) Description of a new dolphin, the Australian snubfin dolphin *Orcaella heinsohni* sp. n. (Cetacean, Delphinidae). *Marine Mammal Science*, **21**, 365–400.

Beerli P (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics*, **22**, 341–345.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 4563–4568.

Beerli P, Palczewski M (2010) Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics*, **185**, 313–326.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Université de Montpellier II, Montpellier.

Bérubé M (2009) Hybridism. In: *Encyclopedia of Marine Mammals*, 2nd edn (eds Perrin W. F., Würsig B., Thewissen J. G. M.), pp. 588–592. Academic Press, San Diego, California.

Bilgmann K, Griffiths O, Allen S, Möller L (2007) A biopsy pole system for bow-riding dolphins: sampling success, behavioral responses and test for sampling bias. *Marine Mammal Science*, **23**, 218–225.

Bilgmann K, Parra GJ, Zanardo N, Beheregaray LB, Möller LM (2014) Multiple management units of short-beaked common dolphins subject to fisheries bycatch off southern and southeastern Australia. *Marine Ecology Progress Series*, **500**, 265–279.

Boness DJ, Clapham PJ, Mesnick SL (2002) Life history and reproductive strategies. In: *Marine Mammal Biology: An Evolutionary Approach* (ed Hoelzel AR), pp. 278–324. Blackwell Science Ltd, Oxford, UK. ISBN 0-632-05232-5.

Brohede J, Ellegren H (1999) Microsatellite evolution: polarity of substitutions within repeats and neutrality of flanking sequences. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **266**, 825–833.

Brown A, Kopps AM, Allen SJ *et al.* (2014) Population differentiation and hybridisation of Australian snubfin (*Orcaella heinsohni*) and Indo-Pacific humpback dolphins (*Sousa chinensis*) in north-western Australia. *PLoS ONE*, **9**, e101427.

Browning NE, McCulloch SD, Bossart GD, Worthy GAJ (2014) Fine-scale population structure of estuarine bottlenose dolphins (*Tursiops truncatus*) assessed using stable isotope ratios and fatty acid signature analyses. *Marine Biology*, **161**, 1307–1317.

Caballero S, Jackson J, Mignucci-Giannoni AA *et al.* (2008) Molecular systematics of South American dolphins *Sotalia*: sister taxa determination and phylogenetic relationships, with insights into a multi-locus phylogeny of the Delphinidae. *Molecular Phylogenetics and Evolution*, **46**, 252–268.

Carwardine M (1995) *Whales, Dolphins and Porpoises*. Dorling Kindersley, London, UK.

Charlton-Robb K, Gershwin L-A, Thompson R, Austin J, Owen K, McKechnie S (2011) A new dolphin species, the Burrunan Dolphin *Tursiops australis* sp. nov., endemic to southern Australian Coastal Waters. *PLoS ONE*, **6**, e24047.

Chilvers BL, Corkeron PJ (2001) Trawling and bottlenose dolphins' social structure. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **268**, 1901–1905.

Cipriano F (1997) Antitropical distributions and speciation in dolphins of the genus *Lagenorhynchus*: a preliminary analysis. In: *Molecular Genetics of Marine Mammals* (eds Dizon A. E., Chivers S. J., Perrin W. F.), pp. 305–316. Soc Mar Mamm, Lawrence, Kansas.

Committee on Taxonomy (2015) List of marine mammal species and subspecies. Society for Marine Mammalogy. www.marinemammalscience.org. Accessed 1 November 2015

Corander JP, Marttinen J, Sirén J, Tang J (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics*, **9**, 539.

Cox TM, Read AJ, Swanner D, Urian K, Waples D (2004) Behavioral responses of bottlenose dolphins, *Tursiops truncatus*, to gillnets and acoustic alarms. *Biological Conservation*, **115**, 203–212.

Cox TM, Lewison RL, Zydelis R, Crowder LB, Safina C, Read AJ (2007) Comparing effectiveness of experimental and implemented bycatch reduction measures: the ideal and the real. *Conservation Biology*, **21**, 1155–1164.

Dixon JM (2008) Fraser's Dolphin. In: *The Mammals of Australia*, 3rd edn (eds Van Dyck S., Strahan R.), p. 847. New Holland Publishers, Sydney, New South Wales.

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Fernández R, Begoña Santos M, Pierce GJ *et al.* (2011) Fine-scale genetic structure of bottlenose dolphins, *Tursiops truncatus*, in Atlantic coastal waters of the Iberian Peninsula. *Hydrobiologia*, **670**, 111–125.

Fletcher WJ, Santoro K (eds) (2013) State of the Fisheries and Aquatic Resources Report 2011/12. Department of Fisheries, Perth. http://www.fish.wa.gov.au/Documents/sofar/status_reports_of_the_fisheries_and_aquatic_resources_2012-13.pdf

Frankham R, Ballou JD, Briscoe DA (2010) *Introduction to Conservation Genetics*, 2nd edn. Cambridge University Press, Cambridge, UK.

Frère CH, Krützen M, Kopps AM, Ward P, Mann J, Sherwin WB (2010) Inbreeding tolerance and fitness costs in wild bottlenose dolphins. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **277**, 2667–2673.

Fruet PF, Secchi ER, Daura-Jorge F *et al.* (2014) Remarkably low genetic diversity and strong population structure in common bottlenose dolphins (*Tursiops truncatus*) from coastal waters of the Southwestern Atlantic Ocean. *Conservation Genetics*, **15**, 879–895.

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**, 305–318.

Geyer CJ (1992) Practical Markov chain Monte Carlo (with discussion). *Statistical Science*, **7**, 473–511.

Gilson A, Syvanen M, Levine K, Banks J (1998) Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. *California Fish and Game*, **84**, 159–169.

Halpern BS, Selkoe KA, Micheli F, Kappel CV (2007) Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. *Conservation Biology*, **21**, 1301–1315.

Hartmann SA, Steyer K, Kraus RHS, Segelbacher G, Nowak C (2013) Potential barriers to gene flow in the endangered European wildcat (*Felis silvestris*). *Conservation Genetics*, **14**, 413–426.

Hassanin A, Delsuc F, Ropiquet A *et al.* (2012) Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. *Comptes Rendus Biologies*, **335**, 32–50.

Hey J (2010) Isolation with migration models for more than two populations. *Molecular Biology and Evolution*, **27**, 905–920.

Hey J, Nielsen R (2007) Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 2785–2790.

Hoelzel AR, Dahlheim M, Stern SJ (1998a) Low genetic variation among killer whales (*Orcinus orca*) in the Eastern North Pacific and genetic differentiation between foraging specialists. *Journal of Heredity*, **89**, 121–128.

Hoelzel AR, Potter CW, Best PB (1998b) Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **265**, 1177–1183.

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, **9**, 1322–1332.

Huelsenbeck JP, Larget B, Alfaro ME (2004) Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. *Molecular Biology and Evolution*, **21**, 1123–1133.

Jaiteh VF, Allen SJ, Meeuwig JJ, Loneragan NR (2013) Subsurface behaviour of bottlenose dolphins (*Tursiops truncatus*) interacting with fish trawl nets in north-western Australia. *Marine Mammal Science* e266. doi:10.1111/j.1748-7692.2012.00620.x

Jaiteh VF, Allen SJ, Meeuwig JJ, Loneragan NR (2014) Combining in-trawl video with observer coverage improves understanding of protected and vulnerable species bycatch in trawl fisheries. *Marine & Freshwater Research*, **65**, 1–8.

Jefferson TA, Webber MA, Pitman RL (2008) *Marine Mammals of the World*. Academic Press, London, UK.

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. *BMC Genetics*, **6**, 13.

Jonker RM, Kraus RHS, Zhang Q *et al.* (2013) Genetic consequences of breaking migratory traditions in barnacle geese *Branta leucopsis*. *Molecular Ecology*, **22**, 5835–5847.

Jost L (2008) GST and its relatives do not measure differentiation. *Molecular Ecology*, **17**, 4015–4026.

Kingston SE, Adams LD, Rosel PE (2009) Testing mitochondrial sequences and anonymous nuclear markers for phylogeny reconstruction in a rapidly radiating group: molecular systematics of the Delphininae (Cetacea: Odontoceti: Delphinidae). *BMC Evolutionary Biology*, **9**, 245.

Kopps AM, Ackermann C, Sherwin WB, Allen SJ, Bejder L, Krützen M (2014) Cultural transmission of tool use combined with habitat specializations leads to fine-scale genetic structure in bottlenose dolphins. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **281**, 20133245.

Kraus RHS, Van Hooft P, Megens H-J *et al.* (2013) Global lack of flyway structure in a cosmopolitan bird revealed by a genome wide survey of single nucleotide polymorphisms. *Molecular Ecology*, **22**, 41–55.

Krützen M, Valsecchi E, Connor RC, Sherwin WB (2001) Characterisation of microsatellites in *Tursiops aduncus*. *Molecular Ecology Notes*, **1**, 170–172.

Krützen M, Barre LM, Möller L, Heithaus MR, Sims C, Sherwin WB (2002) A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Marine Mammal Science*, **18**, 863–878.

Krützen M, Sherwin WB, Berggren P, Gales NJ (2004) Population structure in an inshore cetacean revealed by microsatellite and mtDNA analysis: bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. *Marine Mammal Science*, **20**, 28–47.

Louis M, Viricel A, Lucas T *et al.* (2014) Habitat-driven population structure of bottlenose dolphins, *Tursiops truncatus*, in the North-East Atlantic. *Molecular Ecology*, **23**, 857–874.

Martien KK, Chivers SJ, Baird RW *et al.* (2014) Nuclear and Mitochondrial Patterns of Population Structure in North Pacific False Killer Whales (*Pseudorca crassidens*). *Journal of Heredity*, **105**, 611–626.

McGowen MR (2011) Toward the resolution of an explosive radiation – a multilocus phylogeny of oceanic dolphins (Delphinidae). *Molecular Phylogenetics and Evolution*, **60**, 345–357.

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, **4**, 792–794.

Mendez M, Rosenbaum HC, Wells RS, Stamper A, Bordino P (2010) Genetic evidence highlights potential impacts of bycatch to cetaceans. *PLoS ONE*, **5**, e15550.

Mirimin L, Miller R, Dillane E *et al.* (2011) Fine-scale population genetic structuring of bottlenose dolphins in Irish coastal waters. *Animal Conservation*, **14**, 342–353.

Möller LM, Beheregaray LB (2001) Coastal bottlenose dolphins from South Eastern Australia are *Tursiops aduncus* according to sequences of the mitochondrial DNA control region. *Marine Mammal Science*, **17**, 249–263.

Möller LM, Bilgmann K, Charlton-Robb K, Beheregaray L (2008) Multi-gene evidence for a new bottlenose dolphin species in Southern Australia. *Molecular Phylogenetics and Evolution*, **49**, 674–681.

Moura AE, Nielsen SCA, Vilstrup JT *et al.* (2013) Recent diversification of a marine Genus (*Tursiops* spp.) tracks habitat preference and environmental change. *Systematic Biology*, **62**, 865–877.

Nater A, Kopps AM, Krützen M (2009) New polymorphic tetranucleotide microsatellites improve scoring accuracy in the bottlenose dolphin *Tursiops aduncus*. *Molecular Ecology Resources*, **9**, 531–534.

Natoli A, Peddemors VM, Hoelzel AR (2004) Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. *Journal of Evolutionary Biology*, **17**, 363–375.

Natoli A, Birkun A, Aguilar A, Lopez A, Hoelzel AR (2005) Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **272**, 1217–1226.

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics*, **158**, 885–896.

Oremus M, Poole MM, Steel D, Baker CS (2007) Isolation and interchange among insular spinner dolphin communities in the South Pacific revealed by individual identification and genetic diversity. *Marine Ecology Progress Series*, **336**, 275–289.

Palsbøll PJ, Bérubé M, Aguilar A, Notarbartolo-Di-Sciara G, Nielsen R (2004) Discerning between recurrent gene flow and recent divergence under a finite-site mutation model applied to North Atlantic and Mediterranean Sea fin whale (*Balaenoptera physalus*) populations. *Evolution*, **58**, 670–675.

Palsbøll PJ, Bérubé M, Allendorf FW (2007) Identification of management units using population genetic data. *Trends in Ecology & Evolution*, **22**, 11–16.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics*, **28**, 2537–2539.

Perrin WF, Thieleking JL, Walker WA, Archer FI, Robertson KM (2011) Common bottlenose dolphins (*Tursiops truncatus*) in California waters: cranial differentiation of coastal and offshore ecotypes. *Marine Mammal Science*, **27**, 769–792.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Quérouil S, Silva AM, Freitas L *et al.* (2007) High gene flow in oceanic bottlenose dolphins (*Tursiops truncatus*) of the North Atlantic. *Conservation Genetics*, **8**, 1405–1419.

Read AJ, Drinker P, Northridge S (2006) Bycatch of marine mammals in U.S. and global fisheries. *Conservation Biology*, **20**, 163–169.

Reeves RR, Stewart BS, Clapham PJ, Powell JA (eds) (2002) *Guide to Marine Mammals of the World*. Alfred A. Knopf, New York, NY.

Reeves RR, McClellan K, Werner TB (2013) Marine mammal bycatch in gillnet and other entangling net fisheries, 1990 to 2011. *Endangered Species Research*, **20**, 71–97.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rice DW (1998) *Marine Mammals of the World: Systematics and Distribution*. Soc Mar Mamm, Lawrence, Kansas.

Rosel PE, Dizon AE, Heyning JE (1994) Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). *Marine Biology*, **119**, 159–167.

Ross GJB (2006) *Review of the Conservation Status of Australia's Smaller Whales and Dolphins*. Australian Government, Canberra. 124 p. http://www.environment.gov.au/system/files/pages/e94eb941-2ff5-4a29-89a3-891059be4e47/files/co02conservation-smaller-whales-dolphins.pdf.

Ross HA, Lento GM, Dalebout ML *et al.* (2003) DNA surveillance: web-based molecular identification of whales, dolphins, and porpoises. *Journal of Heredity*, **94**, 111–114.

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Runemark A, Hey J, Hansson B, Svensson EI (2012) Vicariance, divergence and gene flow among islet populations of an endemic lizard. *Molecular Ecology*, **21**, 117–129.

Schaurich M, Vieira Lopes F, de Oliveira L (2012) Hybridization phenomenon in cetacean and pinniped species. *Neotropical Biology and Conservation*, **7**, 199–209.

Sellas AB, Wells RS, Rosel PE (2005) Mitochondrial and nuclear DNA analyses reveal fine scale geographic structure in bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico. *Conservation Genetics*, **6**, 715–728.

Slooten E (2013) Effectiveness of area-based management in reducing bycatch of the New Zealand dolphin. *Endangered Species Research*, **20**, 121–130.

Stephenson PC, Chidlow J (2003) *Bycatch in the Pilbara Trawl Fishery*. Final report to the Natural Heritage Trust. Natural Heritage Trust, Perth, Western Australia. 74 p.

Svane I (2005) Occurrence of dolphins and seabirds and their consumption of by-catch during prawn trawling in Spencer Gulf, South Australia. *Fisheries Research*, **76**, 317–327.

Tezanos-Pinto G, Baker CS, Russell K *et al.* (2009) A worldwide perspective on the population structure and genetic diversity of bottlenose dolphins (*Tursiops truncatus*) in New Zealand. *Journal of Heredity*, **100**, 11–24.

Vilstrup JT, Ho SYW, Foote AD *et al.* (2011) Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae. *BMC Evolutionary Biology*, **11**, 65.

Viricel A, Rosel PE (2012) Evaluating the utility of cox1 for cetacean species identification. *Marine Mammal Science*, **28**, 37–62.

Wade P, Angliss RP (1997) Guidelines for Assessing Marine Mammal Stocks: Report of the GAMMS Workshop April 3-5, 1996, Seattle, WA. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-OPR-12, 93 p.

Wade PR, Reeves RR, Mesnick SL (2012) Social and behavioural factors in cetacean responses to overexploitation: are odontocetes less "resilient" than mysticetes? *Marine Biology*, **2012**, 567276. doi:10.1155/2012/567276.

Wang JY, Chou L-S, White BN (1999) Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus: *Tursiops*) in Chinese waters. *Molecular Ecology*, **8**, 1603–1612.

Wang JY, Chou L-S, White BN (2000a) Osteological differences between two sympatric forms of bottlenose dolphins (genus *Tursiops*) in Chinese waters. *Journal of Zoology*, **252**, 147–162.

Wang JY, Chou L-S, White BN (2000b) Differences in the external morphology of two sympatric species of bottlenose dolphins (genus *Tursiops*) in Chinese waters. *Journal of Mammalogy*, **81**, 1157–1165.

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, **15**, 1419–1439.

Weir B, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wells RS, Rhinehart HL, Cunningham P *et al.* (1999) Long distance offshore movements of bottlenose dolphins. *Marine Mammal Science*, **15**, 1098–1114.

Whitehead H, Reeves RR, Tyack P (2000) Science and the conservation, protection, and management of wild cetaceans. In: *Cetacean Societies: Field Studies of Dolphins and Whales* (eds Mann J., Connor R. C., Tyack P. L., Whitehead H.), pp. 308–332. University of Chicago Press, Chicago, Illinois.

Whitlock MC (2011) G'_{ST} and D do not replace F_{ST}. *Molecular Ecology*, **20**, 1083–1091.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. *Heredity*, **82**, 117–125.

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **163**, 1177–1191.

Wiszniewski J, Allen SJ, Möller LM (2009) Social cohesion in a hierarchically structured embayment population of Indo-Pacific Bottlenose Dolphins. *Animal Behaviour*, **77**, 1449–1457.

Wiszniewski J, Beheregaray LB, Allen SJ, Möller LM (2010) Environmental and social influences on the genetic structure of bottlenose dolphins (*Tursiops aduncus*) of South-Eastern Australia. *Conservation Genetics*, **11**, 1405–1419.

Woinarski JCZ, Burbidge AA, Harrison PL (2014) *The Action Plan for Australian Mammals 2012*. CSIRO Publishing, Collingwood, Victoria.

Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.

Wright S (1943) Isolation by distance. *Genetics*, **28**, 114–138.
Xia X, Xie Z (2001) DAMBE: data analysis in molecular biology and evolution. *Journal of Heredity*, **92**, 371–373.
Xiong Y, Brandley MC, Xu S, Zhou K, Yang G (2009) Seven new dolphin mitochondrial genomes and a time-calibrated phylogeny of whales. *BMC Evolutionary Biology*, **9**, 20.
Yang G, Guoqing J, Ren W, Zhou K (2005) Pattern of genetic variation of bottlenose dolphins in Chinese waters. *Raffles Bulletin of Zoology*, **53**, 157–164.
Zhou X, Xu S, Yang Y, Zhou K, Yang G (2011) Phylogenomic analyses and improved resolution of Cetartiodactyla. *Molecular Phylogenetics and Evolution*, **61**, 255–264.

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Data accessibility

Data for this manuscript have been made available on DRYAD: doi:10.5061/dryad.908g4.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Sample vouchers from Genbank, species and corresponding references used to provide comparison with the samples collected here.

Table S2 Genetic diversity indices for sampling sites for all 19 microsatellite loci.

Data S1 Recoded microsatellite data and input file for ImA analysis.

Data S2 Input file for migrate analysis.

Data S3 Input file for mtDNA analysis.

Data S4 IBD analysis.

Report

Current Biology

Bottlenose Dolphins Retain Individual Vocal Labels in Multi-level Alliances

Highlights

- Individual vocal labels play a central role in the maintenance of nested alliances
- Male dolphins in nested alliances retain vocal labels distinct from their allies
- Motor synchrony and not vocal convergence is used to indicate social proximity

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In Brief

Vocal convergence is frequently used to signal social proximity between individuals. King et al. show that multi-level dolphin alliances do not converge onto shared calls but retain individual vocal labels. This suggests that vocal labels play a central role in the recognition of cooperative partners and competitors in complex biological markets.

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Current Biology
Report

Bottlenose Dolphins Retain Individual Vocal Labels in Multi-level Alliances

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SUMMARY

Cooperation between allied individuals and groups is ubiquitous in human societies, and vocal communication is known to play a key role in facilitating such complex human behaviors [1, 2]. In fact, complex communication may be a feature of the kind of social cognition required for the formation of social alliances, facilitating both partner choice and the execution of coordinated behaviors [3]. As such, a compelling avenue for investigation is what role flexible communication systems play in the formation and maintenance of cooperative partnerships in other alliance-forming animals. Male bottlenose dolphins in some populations form complex multi-level alliances, where individuals cooperate in the pursuit and defense of an important resource: access to females [4]. These strong relationships can last for decades and are critical to each male's reproductive success [4]. Convergent vocal accommodation is used to signal social proximity to a partner or social group in many taxa [5, 6], and it has long been thought that allied male dolphins also converge onto a shared signal to broadcast alliance identity [5–8]. Here, we combine a decade of data on social interactions with dyadic relatedness estimates to show that male dolphins that form multi-level alliances in an open social network retain individual vocal labels that are distinct from those of their allies. Our results differ from earlier reports of signature whistle convergence among males that form stable alliance pairs. Instead, they suggest that individual vocal labels play a central role in the maintenance of differentiated relationships within complex nested alliances.

RESULTS AND DISCUSSION

Animals that form strong social bonds tend to vocally accommodate one another by converging onto shared calls [5, 6]. Convergent vocal accommodation is used to signal social proximity to a partner or social group [5, 6, 9] and has been well documented in a variety of birds and non-human mammals, such as chickadees [10], parrots [11], bats [12], primates [13, 14], and elephants [15]. Phonetic convergence in humans has also been linked to relationship strength, where stronger bonds lead to a higher degree of convergence [16]. Further, convergence onto shared or similar identity signals has been documented in allied male bottlenose dolphins (both *Tursiops aduncus* and *T. truncatus*) [7, 8].

Bottlenose dolphins are adept vocal production learners, a notably rare skill in mammals [17], and use vocal learning to develop their individually specific signature whistle, which they use to broadcast their identity [18]. Signature whistles are developed within the first few months of an individual's life and are structurally unique from those of conspecifics [17, 19]. The pervasive notion that alliance partners will converge onto a shared signature [6–8] is perhaps surprising, given that the signature whistle is a rare example of a non-human mammal using a learned vocal label that can be considered somewhat comparable to a human name [20]. However, suggested benefits of "alliance signatures" include broadcasting alliance identity as a specific social unit toward other allied males or to sexually receptive females [6, 8]. Although one study showed that allied males tend to have signature whistles that are more similar to their partner's than to non-partners [7], this finding was based purely on dyadic relationships. In Shark Bay, Western Australia, males cooperate together in pairs or trios, known as first-order alliances, to sequester and control the movements of single oestrous females [4]. Each male, in turn, belongs to a second-order alliance of 4–14 males, considered the core unit of male social organization, who work together to acquire and defend females [4]. Whistle convergence was previously documented among males in Shark Bay, but the study was limited to one trio in an unusual recording context and did not consider partnerships outside this first-order alliance [8]. Thus, the influence of

Table 1. Summary of the 17 Adult Males Used in this Study, Their Second-Order Alliance Membership, Mean Association Coefficient, i.e., CoA, for Their First-Order Alliance, Their Age in Years, and the Number of Signature Whistles Recorded for Each Male across Days and Years

First-Order Alliance Mean CoA	Individual Male ID Code	Age (Years)	No. Signature Whistles Recorded (Days/Years)
Second-Order Alliance ID Code: KS			
0.7	PON	est. >30	34 (8/3)
	QUA[a]	29	20 (3/2)
	PAS	32	64 (6/4)
0.5[b]	CEB	31	35 (11/4)
	MOG	est. >30	26 (3/2)
	DEE	31	30 (5/3)
	IMP	est. >30	24 (8/3)
0.33	NOG[a,c]	est. >30	22 (1/1)
	DNG	32	31 (3/3)
Second-Order Alliance ID Code: PD			
0.76	RID	est. >30	60 (7/3)
	FRE	est. >30	32 (7/5)
	BIG	est. >30	12 (3/2)
0.88	NAT	est. >30	36 (6/4)
	WAB[c]	est. >30	24 (3/3)
Second-Order Alliance ID Code: RR			
0.65	COO	29	40 (6/3)
	SMO	29	36 (4/2)
	URC	27	36 (5/3)

All males have significant home range overlap [4] and frequently interact with one another. See also Figure S1.

[a]QUA not seen in 2017; NOG not seen after 2013.

[b]Only three of these males consort together at any one time, but consorting partners changed frequently among the four.

[c]Signature whistles confirmed by exclusion, where all other signature whistles within the first-order alliance had been localized to other individual males (STAR Methods).

nested alliance relationships on whistle similarity between cooperative partners remains unknown. Here, we investigated signature whistle convergence in first- and second-order alliances in Shark Bay, Western Australia, where our long-term dolphin research project has been conducted on a seasonal basis since 1982. We collected focal follow data on allied males and used acoustic localization and the SIGID (signature identification) method [21] to identify individual signature whistles. We also used long-term photo-identification records to determine the strength of alliance associations (calculated over a 10-year period) and single-nucleotide polymorphisms (SNPs) to estimate dyadic relatedness between males. These analyses were carried out in order to determine whether strong social bonds and/or genetic relatedness influence whistle similarity between cooperative partners.

Signature Whistle Similarity between Alliance Partners

We identified the signature whistles of 17 individual adult males that comprise six first-order alliances across three different second-order alliances (Table 1; Figure 1; see Figure S1 for determination of alliance membership). The majority of signature whistles were confirmed using acoustic localization, with the exception of two whistles that were confirmed by exclusion (see STAR Methods), where the whistles of all other alliance partners were known (Table 1). Whistle similarity between allied and non-allied males was quantified using two methods: visual classification by human judges [7, 20] and a dynamic time warp analysis [22]. The 12 human judges (blind to context and identity) showed substantial inter-observer agreement in their signature whistle similarity scores (mean weighted kappa statistic: 0.7, $p < 0.0001$).

Visual Classification

We found no evidence of whistle convergence, with mixed-effect models detecting no effect of social relationship strength and/or genetic relatedness on whistle similarity (Figure 2A; Data S1). Furthermore, similarity scores of ≥ 3, which indicate higher levels of similarity, were more common between males in different second-order alliances than within alliances (Figure 2B). Thus, allied males tended to have signature whistles that were less similar to their alliance partners, with only one male (MOG) found to have the highest similarity score solely with a first-order partner (Figure 2).

Dynamic Time Warp Analysis

The dynamic time warp approach allowed us to expand from a single model signature whistle per male to a set of ten signature whistles per male. Pairwise dissimilarities were relatively consistent across whistle replicates, with few exceptions (Figure S2). Similar to the visual classification analysis, mixed-effect models detected no effect of social relationship strength and/or genetic relatedness on dynamic time warp whistle similarity (Figure 3A; Data S2). Allied males did not have signature whistles that were more similar to their alliance partners. In fact, the mean similarity for first- and second-order alliance partners was no different from the mean similarity between males from different second-order alliances (Figure 3B). The most similar signature whistle was found predominantly (13 of 17 cases) in males from different second-order alliances, and, again, in only one case did an individual have the most similar signature whistle with his first-order alliance partner (Figure 3C).

Conclusions

We detected no evidence of signature whistle convergence between cooperative partners in nested bottlenose dolphin (*T. aduncus*) alliances. Our results differ from prior research, which suggested that closely affiliated male dolphins produce similar signature whistles [7, 8]. We also found no evidence of genetic relatedness influencing signature whistle similarity between males. Most of the males in this study had signature whistles that were notably different from those of both first- and second-order alliance partners. Our findings therefore suggest that individual vocal labels, rather than shared identity calls, play a central role in maintaining recognition within complex nested alliances.

The lack of a genetic influence on whistle similarity between males is unsurprising, given that signature whistle development is strongly influenced by vocal learning [17]. The fact that many of

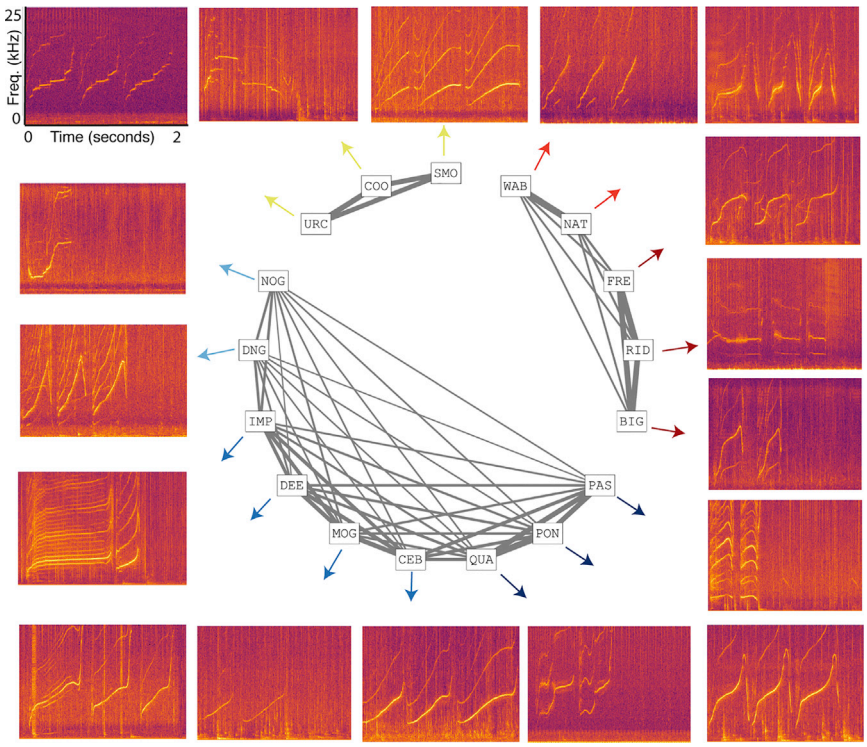

Figure 1. Social Network of 17 Adult Males Grouped in Their Second-Order Alliances

Only coefficients of association (CoAs) ≥ 0.2 are shown, as this reliably identifies second-order alliance partners, and males are color coded by alliance membership. The thickness of the lines indicates the strength of the dyadic social relationship, and alongside each male is a spectrogram of his signature whistle (sampling rate: 96 kHz; fast Fourier transform [FFT] length: 1,024; Hanning window function). See also Figure S1 for determination of alliance membership.

the allied males in our study have signature whistles with low similarity scores is most likely a result of differences in their early acoustic and social environments. Of the seven dyads in our study that were first sighted together when still dependent calves, none had a visual whistle similarity score greater than 2 (mean = 1.6). There is some evidence to suggest that calves develop signature whistles that resemble those of relatively rare associates of their mothers [19]. Therefore, male calves of mothers with strong associations would be expected to develop whistles with low similarity. Furthermore, the nine dyads in our study that had relatively high visual whistle similarity scores (≥ 3) were first sighted together as sub-adults (mean: 9.5 years, range: 3–15 years), i.e., when their signature whistles were already developed. The majority of their individual sightings histories pre-date their first joint sighting. Although one of those dyads consisted of first-order alliance partners, this is no more than expected by chance, with our broader results demonstrating a striking lack of convergence.

Our findings differ from the original study on whistle convergence that was conducted on three male dolphins within the Shark Bay population [8]. These males formed an alliance over a 4-year period and appeared to have converged onto one shared whistle type [8]. However, their alliance formed under abnormal conditions where the recordings were obtained, i.e., in shallow water by a beach where humans regularly provisioned them with fish. The small sample size and unusual context may explain their findings. Although our research clearly demonstrates the lack of long-term vocal convergence in signature whistles between adult male dolphins in Shark Bay, it provides only a snapshot of existing alliances over the duration of the study. For example, we were unable to determine whether these signature whistles had been modified during the lifetime of each male. However, at least one adult male (COO) in an established alliance uses the same signature whistle first recorded when he was an infant (1.5 years of age) over a quarter of a century ago [23], supporting the notion that signature stability in males can span decades, as it can in females [24].

Interestingly, allied pairs of common bottlenose dolphins (*T. truncatus*) in Sarasota Bay, Florida, do tend to have signature whistles that are similar in structure [7]. Selection may favor the convergence of such calls within a dyad if there is only one partner to vocally accommodate. In that instance, convergence between individuals within a pair may function in signaling their commitment to one another, as shown for avian duetting [25]. However, in a society in which nested alliances are formed, where males within second-order alliances show notable differences in partner preferences [4] and first-order alliance stability can vary [4], there may be no adaptive benefit to signature whistle convergence. Instead, there appears to be a strong benefit in retaining an individual signature whistle that is distinct from one's allies.

Vocal accommodation in humans has been suggested as serving as a phenotypic "tag" for cooperation, where the convergence of dialects facilitates cooperation between individuals [5, 26, 27]. The lack of a relationship between social proximity and vocal similarity in our study would suggest that such tags are not required for cooperation. However, it should be noted that whereas human children can acquire new dialects, accommodation in adults involves only subtle shifts and rarely leads to completely new dialect acquisition [5]. As such, speech accommodation during short dyadic interactions can promote social identity between individuals [5, 16], but there is no evidence of long-term convergence of identity signals in humans. In fact, the ability of individuals to have control over with whom they cooperate plays an important role in stabilizing large-scale cooperation in human societies [28]. The structure of social networks can promote choosiness and a need to monitor the behavior of others to optimize partner choice [29]. This places a demand on the recognition of a large number of individuals and their third-party relationships with other conspecifics [30]. Thus, in those species that form nested alliances, individual vocal labels may reliably facilitate the recognition of many

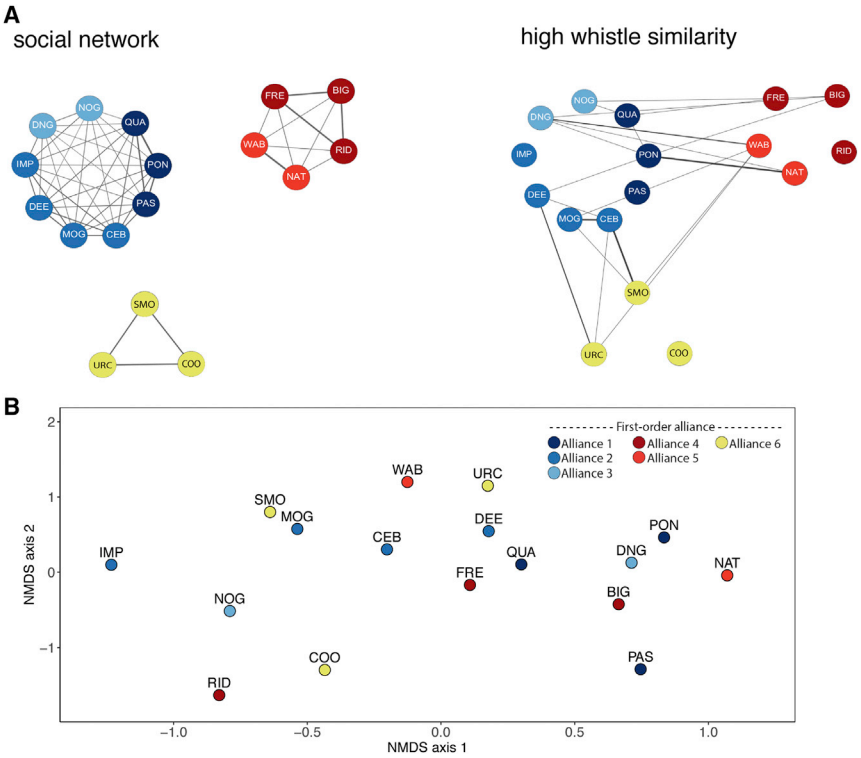

Figure 2. Analysis of Whistle Similarity Based on Visual Classification

(A) Network plots of the three second-order alliances, with males color coded by alliance membership; the left network shows pairwise relationships between males with CoAs $\geq$0.2, and the right network plot shows the pairwise median similarity scores that are $\geq$3 from the visual classification.

(B) Non-linear multidimensional scaling of the pairwise whistle similarity scores.

See also Data S1 and Table S1.

cooperative partners and competitors in complex biological markets [31]. Indeed, the bottlenose dolphin's propensity for the use of learned vocal labels [20] and long-term social recognition [32] may well have enabled the formation of their nested alliances.

In contrast to our study system, many non-human animals converge on group distinctive identity calls as a means of promoting group cohesion and strengthening social bonds [6]. So, under what evolutionary conditions is the convergence of identity calls favored? The study of animal populations with similar phylogenetic traits to those of the Shark Bay dolphins, such as fission-fusion social systems, vocal flexibility, and long-term social memory, may shed light on this important question. For example, under certain conditions, it appears that the importance of individual vocal labels in forming and maintaining cooperative strategies may well take precedence over any conferred benefits of vocal convergence. If that is the case, then other affiliative strategies are required to indicate social proximity.

The two obvious mechanisms for mediating social proximity between male dolphins in Shark Bay are affiliative tactile contact and synchrony [33]. Males mediate alliance relationships with gentle contact behaviors, such as petting, as a means of maintaining their strong male-male bonds, similar to primate grooming [30, 33, 34]. In chimpanzees, grooming between partners with strong social bonds has been directly linked to oxytocin release [35], and the role of oxytocin in facilitating bonding between humans and other animals has been well documented

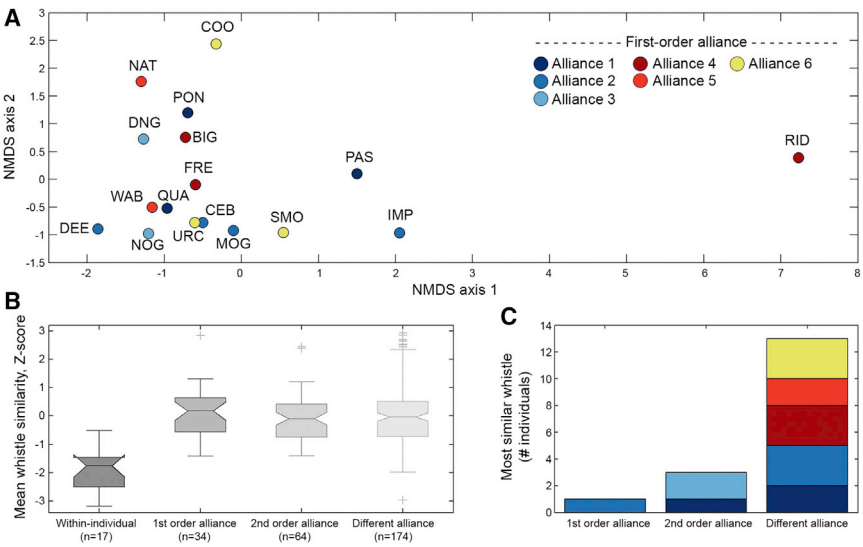

Figure 3. Analysis of Whistle Similarity Based on Dynamic Time Warping

(A) Non-linear multidimensional scaling of pairwise dissimilarity values.

(B) Mean whistle similarity (dissimilarity values were log transformed and then standardized to *Z* scores for each individual before pooling) according to alliance membership. Note that the categories are exclusive so that pairs in the second-order alliance category are not from the same first-order alliance.

(C) Number of individuals where the male with the most similar signature whistle was a first- or second-order alliance partner or in a different alliance. Colors represent first-order alliance membership.

See also Data S2, Figure S2, and Table S2.

[36, 37]. Increased oxytocin release has also been linked to social synchrony in humans [38], promoting trust [37], cooperation [36], and social bonding [39]. Synchronous behavior may therefore have evolved as a coalition signaling system in human societies to indicate the quality of the cooperative relationship [40]. We know that synchrony also plays an important role in affiliative interactions between male dolphins in Shark Bay [30]. In fact, it is synchrony, rather than shared identity calls, that functions as a signal of unity [30, 33], representing convergence with humans in the use of synchrony to promote both cooperation and coordination between allied males [36]. Thus, nested alliances in dolphins appear to be similar to those in humans, in which synchrony is an adaptive signal indicating the quality of relationships [33, 40], but recognition is maintained through individual vocal labels or 'names'.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, three tables, and two data files and can be found with this article online at https://doi.org/10.1016/j.cub.2018.05.013.

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AUTHOR CONTRIBUTIONS

S.L.K. conceived the study, acquired funding, collected data, conducted the analysis and drafted the manuscript. M.K., R.C.C., S.J.A., and W.R.F. acquired funding. W.R.F., S.J.A., L.G., S.W., R.C.C., and M.K. significantly contributed to data collection. M.K., L.G., and S.W. conducted the genetic analysis. F.J. conducted the dynamic time warping analysis. All authors edited the manuscript, provided critical review, and gave final approval for submission.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Boyd, R., and Richerson, P.J. (2009). Culture and the evolution of human cooperation. Philos. Trans. R. Soc. Lond. B Biol. Sci. *364*, 3281–3288.
2. Smith, E.A. (2010). Communication and collective action: language and the evolution of human cooperation. Evol. Hum. Behav. *31*, 231–245.
3. McAuliffe, K., and Thornton, A. (2015). The psychology of cooperation in animals: an ecological approach. J. Zool. (Lond.) *295*, 23–35.
4. Connor, R.C., and Krützen, M. (2015). Male dolphin alliances in Shark Bay: changing perspectives in a 30-year study. Anim. Behav. *103*, 223–235.
5. Ruch, H., Zürcher, Y., and Burkart, J.M. (2017). The function and mechanism of vocal accommodation in humans and other primates. Biol. Rev. Camb. Philos. Soc. *93*, 996–1013.
6. Tyack, P.L. (2008). Convergence of calls as animals form social bonds, active compensation for noisy communication channels, and the evolution of vocal learning in mammals. J. Comp. Psychol. *122*, 319–331.
7. Watwood, S.L., Tyack, P.L., and Wells, R.S. (2004). Whistle sharing in paired male bottlenose dolphins, *Tursiops truncatus*. Behav. Ecol. Sociobiol. *55*, 531–543.
8. Smolker, R., and Pepper, J.W. (1999). Whistle convergence among allied male bottlenose dolphins (*Delphinidae Tursiops sp.*). Ethology *105*, 595–617.
9. Giles, H., Coupland, J., and Coupland, N. (1991). Accommodation theory: communication, context, and consequence. In Contexts of Accommodation: Developments in Applied Sociolinguistics, H. Giles, J. Coupland, and N. Coupland, eds. (Cambridge University Press), pp. 1–68.
10. Mammen, D.L., and Nowicki, S. (1981). Individual differences and within-flock convergence in chickadee calls. Behav. Ecol. Sociobiol. *9*, 179–186.
11. Scarl, J.C., and Bradbury, J.W. (2009). Rapid vocal convergence in an Australian cockatoo, the galah *Eolophus roseicapillus*. Anim. Behav. *77*, 1019–1026.
12. Boughman, J.W. (1997). Greater spear-nosed bats give group distinctive calls. Behav. Ecol. Sociobiol. *40*, 61–70.
13. Mitani, J.C., and Gros-Louis, J. (1998). Chorusing and call convergence in chimpanzees: tests of three hypotheses. Behaviour *135*, 1041–1064.
14. Watson, S.K., Townsend, S.W., Schel, A.M., Wilke, C., Wallace, E.K., Cheng, L., West, V., and Slocombe, K.E. (2015). Vocal learning in the functionally referential food grunts of chimpanzees. Curr. Biol. *25*, 495–499.
15. Poole, J.H., Tyack, P.L., Stoeger-Horwath, A.S., and Watwood, S. (2005). Animal behaviour: elephants are capable of vocal learning. Nature *434*, 455–456.
16. Pardo, J.S., Gibbons, R., Suppes, A., and Krauss, R.M. (2012). Phonetic convergence in college roommates. J. Phonetics *40*, 190–197.
17. Janik, V.M. (2014). Cetacean vocal learning and communication. Curr. Opin. Neurobiol. *28*, 60–65.
18. Caldwell, M.C., Caldwell, D.K., and Tyack, P.L. (1990). Review of the signature whistle hypothesis for the Atlantic bottlenose dolphin. In The Bottlenose Dolphin, S. Leatherwood, and R. Reeves, eds. (Academic Press), pp. 199–234.

19. Fripp, D., Owen, C., Quintana-Rizzo, E., Shapiro, A., Buckstaff, K., Jankowski, K., Wells, R., and Tyack, P. (2005). Bottlenose dolphin (*Tursiops truncatus*) calves appear to model their signature whistles on the signature whistles of community members. Anim. Cogn. *8*, 17–26.

20. King, S.L., and Janik, V.M. (2013). Bottlenose dolphins can use learned vocal labels to address each other. Proc. Natl. Acad. Sci. USA *110*, 13216–13221.

21. Janik, V., King, S., Sayigh, L., and Wells, R. (2013). Identifying signature whistles from recordings of groups of unrestrained bottlenose dolphins (*Tursiops truncatus*). Mar. Mamm. Sci. *29*, 109–122.

22. Buck, J.R., and Tyack, P.L. (1993). A quantitative measure of similarity for *tursiops truncatus* signature whistles. J. Acoust. Soc. Am. *94*, 2497–2506.

23. Smolker, R.A., Mann, J., and Smuts, B.B. (1993). Use of signature whistles during separations and reunions by wild bottlenose dolphin mothers and infants. Behav. Ecol. Sociobiol. *33*, 393–402.

24. Sayigh, L.S., Tyack, P.L., Wells, R.S., and Scott, M.D. (1990). Signature whistles of free-ranging bottlenose dolphins (*Tursiops truncatus*): stability and mother-offspring comparisons. Behav. Ecol. Sociobiol. *26*, 247–260.

25. Hall, M.L. (2004). A review of hypotheses for the functions of avian duetting. Behav. Ecol. Sociobiol. *55*, 415–430.

26. Cohen, E. (2012). The evolution of tag-based cooperation in humans. Curr. Anthropol. *53*, 588–616.

27. Antal, T., Ohtsuki, H., Wakeley, J., Taylor, P.D., and Nowak, M.A. (2009). Evolution of cooperation by phenotypic similarity. Proc. Natl. Acad. Sci. USA *106*, 8597–8600.

28. Rand, D.G., Arbesman, S., and Christakis, N.A. (2011). Dynamic social networks promote cooperation in experiments with humans. Proc. Natl. Acad. Sci. USA *108*, 19193–19198.

29. McNamara, J.M., and Leimar, O. (2010). Variation and the response to variation as a basis for successful cooperation. Philos. Trans. R. Soc. Lond. B Biol. Sci. *365*, 2627–2633.

30. Connor, R.C. (2007). Dolphin social intelligence: complex alliance relationships in bottlenose dolphins and a consideration of selective environments for extreme brain size evolution in mammals. Philos. Trans. R. Soc. Lond. B Biol. Sci. *362*, 587–602.

31. Barclay, P. (2016). Biological markets and the effects of partner choice on cooperation and friendship. Curr. Opin. Psychol. *7*, 33–38.

32. Bruck, J.N. (2013). Decades-long social memory in bottlenose dolphins. Proc. Biol. Sci. *280*, 20131726.

33. Connor, R.C., Smolker, R., and Bejder, L. (2006). Synchrony, social behaviour and alliance affiliation in Indian Ocean bottlenose dolphins, *Tursiops truncatus*. Anim. Behav. *72*, 1371–1378.

34. Patzelt, A., Kopp, G.H., Ndao, I., Kalbitzer, U., Zinner, D., and Fischer, J. (2014). Male tolerance and male-male bonds in a multilevel primate society. Proc. Natl. Acad. Sci. USA *111*, 14740–14745.

35. Crockford, C., Wittig, R.M., Langergraber, K., Ziegler, T.E., Zuberbühler, K., and Deschner, T. (2013). Urinary oxytocin and social bonding in related and unrelated wild chimpanzees. Proc. Biol. Sci. *280*, 20122765.

36. De Dreu, C.K.W., Greer, L.L., Handgraaf, M.J.J., Shalvi, S., Van Kleef, G.A., Baas, M., Ten Velden, F.S., Van Dijk, E., and Feith, S.W.W. (2010). The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. Science *328*, 1408–1411.

37. Kosfeld, M., Heinrichs, M., Zak, P.J., Fischbacher, U., and Fehr, E. (2005). Oxytocin increases trust in humans. Nature *435*, 673–676.

38. Spengler, F.B., Scheele, D., Marsh, N., Kofferath, C., Flach, A., Schwarz, S., Stoffel-Wagner, B., Maier, W., and Hurlemann, R. (2017). Oxytocin facilitates reciprocity in social communication. Soc. Cogn. Affect. Neurosci. *12*, 1325–1333.

39. Launay, J., Tarr, B., and Dunbar, R.I.M. (2016). Synchrony as an adaptive mechanism for large-ccale human social bonding. Ethology *122*, 779–789.

40. Hagen, E.H., and Bryant, G.A. (2003). Music and dance as a coalition signaling system. Hum. Nat. *14*, 21–51.

41. Whitehead, H. (2009). SOCPROG programs: analysing animal social structures. Behav. Ecol. Sociobiol. *63*, 765–778.

42. Bastian, M., Heymann, S., and Jacomy, M. (2009). Gephi: an open source software for exploring and manipulating networks. In International AAAI Conference on Weblogs and Social Media.

43. Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat. Methods *9*, 357–359.

44. Van der Auwera, G.A., Carneiro, M.O., Hartl, C., Poplin, R., del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., et al. (2013). From fastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. Curr. Protoc. Bioinformatics *43*, 11.10.1–11.10.33.

45. Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., et al.; 1000 Genomes Project Analysis Group (2011). The variant call format and VCFtools. Bioinformatics *27*, 2156–2158.

46. Wang, J. (2011). COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol. Ecol. Resour. *11*, 141–145.

47. O'Leary, N.A., Wright, M.W., Brister, J.R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., et al. (2016). Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. *44* (D1), D733–D745.

48. Quick, N.J., Rendell, L.E., and Janik, V.M. (2008). A mobile acoustic localisation system for the study of free-ranging dolphins during focal follows. Mar. Mamm. Sci. *24*, 979–989.

49. Wahlberg, M., Møhl, B., and Madsen, P.T. (2001). Estimating source position accuracy of a large-aperture hydrophone array for bioacoustics. J. Acoust. Soc. Am. *109*, 397–406.

50. Schulz, T.M., Rendell, L.E., and Whitehead, H. (2006). A remotely-piloted acoustic array for studying sperm whale vocal behaviour. Can. Acoust. *34*, 54–55.

51. Smolker, R.A., Richards, A.F., Connor, R.C., and Pepper, J.W. (1992). Sex differences in patterns of association among Indian Ocean bottlenose dolphins. Behaviour *123*, 38–69.

52. Hoppitt, W.J.E., and Farine, D.R. (2017). Association indices for quantifying social relationships: how to deal with missing observations of individuals or groups. Anim. Behav. *136*, 227–238.

53. Ginsberg, J.R., and Young, T.P. (1992). Measuring association between individuals or groups in behavioural studies. Anim. Behav. *44*, 377–379.

54. Deecke, V.B., and Janik, V.M. (2006). Automated categorization of bioacoustic signals: avoiding perceptual pitfalls. J. Acoust. Soc. Am. *119*, 645–653.

55. Quick, N.J., and Janik, V.M. (2012). Bottlenose dolphins exchange signature whistles when meeting at sea. Proc. Biol. Sci. *279*, 2539–2545.

56. Janik, V.M. (2000). Whistle matching in wild bottlenose dolphins (*Tursiops truncatus*). Science *289*, 1355–1357.

57. Sayigh, L.S., Tyack, P.L., Wells, R.S., Scott, M.D., and Irvine, A.B. (1995). Sex difference in signature whistle production of free-ranging bottlenose dolphins, *Tursiops truncatus*. Behav. Ecol. Sociobiol. *36*, 171–177.

58. Cohen, J. (1968). Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. Psychol. Bull. *70*, 213–220.

59. Janik, V.M., Sayigh, L.S., and Wells, R.S. (2006). Signature whistle shape conveys identity information to bottlenose dolphins. Proc. Natl. Acad. Sci. USA *103*, 8293–8297.

60. Esch, H.C., Sayigh, L.S., and Wells, R.S. (2009). Quantifying parameters of bottlenose dolphin signature whistles. Mar. Mamm. Sci. *24*, 976–986.

61. Ralston, J.V., and Herman, L.M. (1995). Perception and generalization of frequency contours by a bottlenose dolphin (*Tursiops truncatus*). J. Comp. Psychol. *109*, 268–277.

62. Krutzen, M., Barre, L.M., Moller, L.M., Heithaus, M.R., Simms, C., and Sherwin, W.B. (2002). A biopsy system for small cetaceans: darting success and wound healing in Tursiops SPP. Mar. Mamm. Sci. *18*, 863–878.

63. Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., and Hoekstra, H.E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE *7*, e37135.

64. Wang, J. (2007). Triadic IBD coefficients and applications to estimating pairwise relatedness. Genet. Res. *89*, 135–153.

65. Nakagawa, S., and Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. *4*, 133–142.

66. Legendre, P., and Legendre, L. (2012). Numerical Ecology, Third Edition (Elsevier).

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Tursiops aduncus	Shark Bay, Western Australia	Prof M. Krützen, University of Zürich.
Software and Algorithms		
SOCPROG	[41]	http://whitelab.biology.dal.ca/SOCPROG/social.htm
R	The R Foundation	http://www.r-project.org; RRID:SCR_001905
Gephi	[42]	https://gephi.org/; RRID:SCR_004293
MATLAB	MathWorks	https://www.mathworks.com; RRID:SCR_001622
Bowtie2	[43]	http://bowtie-bio.sourceforge.net/bowtie2
Genome Analysis Toolkit GATK	[44]	https://software.broadinstitute.org/gatk/; RRID:SCR_001876
Vcftools	[45]	http://vcftools.sourceforge.net/; RRID:SCR_001235
Coancestry	[46]	https://www.zsl.org/science/software/coancestry
Other		
T. truncatus reference assembly GenBank: GCA_001922835.1	[47]	https://www.ncbi.nlm.nih.gov/assembly/GCF_001922835.1/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Stephanie King (stephanie.king@uwa.edu.au).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

We worked with free-ranging adult male bottlenose dolphins (*Tursiops aduncus*) in the eastern gulf of Shark Bay, Western Australia, where our long-term dolphin research project has been running on a seasonal basis (typically austral winter and spring) since 1982. Males ranged from approximately 27 to 40 years old. Permits for the scientific use of animals were obtained from the Department of Biodiversity, Conservation and Attractions (DBCA), Western Australia. The University of Zurich and University of Western Australia granted animal ethics approvals.

METHOD DETAILS

Acoustic data collection

Acoustic data were collected during focal behavioral follows of allied male dolphins between August and September 2016 and June to September 2017. Focal follows were conducted from a 5.4 m research vessel using a towed hydrophone array consisting of four HTI-96 MIN series (flat frequency response: 0.002–30 kHz ± 1 dB) in a similar configuration to Quick et al. [48]. Recordings were made onto a TASCAM DR-680 MKII multi-track recorder at a sampling rate of 96 kHz. A spoken track was used to note the bearing (compass bearing, where the boat's bow is 0°), distance (m) and identification of the focal animals at each surfacing. Aerial video was also used to document animal movement and relative position, with the use of a GoPro Hero4 attached to a 1 m^3 Allsopp Skyshot Helikite, which was attached to the bow of the boat using flying line and an Okuma Solterra Game Fishing Reel and flown at an altitude of c. 30 m. The aerial video allowed us to simultaneously record the movements, including some subsurface movements, of multiple individuals over much larger distances than visual observations from the research vessel allowed. The aerial video data also assisted in the interpretation of the acoustic localization.

Individual dolphins were identified by trained observers on the research vessel via their unique dorsal fins, and corroborated with photo-identification data collected using a Canon 50D camera and 100-400 mm IS lens. Group composition was verified every five mins and all changes in group composition were recorded *ad lib* during focal follows; these data were synchronized to recordings prior to analysis. The engine was switched off during recordings and only whistles with a good signal to noise ratio were used for localization. Localization error of the array was calculated using custom-written MATLAB routines to calculate 2D averaged MINNA (minimum number of receiver array) localizations using the methods described in Wahlberg et al. [49] and Schulz et al. [50].

The array was calibrated using two different frequency modulated dolphin whistles, each approximately 1.5 s in duration with a frequency range of 4-20kHz. Acoustic localization errors for directions (n = 75) were calculated as 76% within ± 15 degrees, and 99% within ± 30 degrees.

Additional acoustic data were collected during focal follows of allied males between August and November 2013 and September and November 2014, using a single towed hydrophone designed by the Scripps Whale Acoustics Lab at the University of California, San Diego. The hydrophone was equipped with a low-frequency transducer (flat frequency response: 0.4–15 kHz ± 3 dB) and a high-frequency transducer (flat frequency response: 15 kHz–120 kHz ± 8 dB) with a notch at 25 kHz, which were summed before digitising. Recordings were made onto a Fostex FR-2 memory recorder at a sampling rate of 192 kHz. Animal identity and group composition data collection followed the methods described above. These single hydrophone data assisted in characterizing the whistle repertoire of our focal alliances.

Behavioral data collection: Strength of male relationships

Survey data were used to calculate association indices between pairs of males. A ''survey'' is a minimum five-min observation of dolphin group composition (''group'' being defined by the 10-m 'chain rule') and behavioral activity [51]. Survey data are collected annually as part of our long-term research program, with behavioral survey data spanning 35 years. For this study, pairwise Coefficients of Association (CoA) were calculated over a 10-year period using SOCPROG 2.7 [41] and the Simple Ratio Index (SRI). The SRI is an estimate of the proportion of time two animals spend together (0 for pairs of animals that never associate; 1 for pairs always seen together) [52, 53]. CoAs were calculated using the last 10 years of survey data for each male prior to it last being seen alive (two males disappeared during our study). The sampling period was day and only association data recorded in the first five mins of a survey were used. Restriction to the use of just the first 5 min of observation ensured that association measures were comparable across all surveys.

To confirm that a CoA cut-off value of 0.2 reliably identified second-order alliance partners, we conducted a changepoint analysis using the Pruned Exact Linear Time (PELT) method (*changepoint package* in R) on the SRI coefficients of 66 adult males in eastern Shark Bay. Only CoA values greater than zero were used in the analysis. The first changepoint occurred at a SRI coefficient of 0.2, a cut-off value that is in line with previous studies [4, 51]. We therefore used $\geq$ 0.2 as a cut-off for second-order allies, and first-order allies were based on hierarchical clustering with coefficients ranging from 0.33 to 0.88 (Figure S1). All allied males in this study were also frequently observed consorting females together, thus alliances were defined not only by their association indices, but also their functional behavior. All social network figures were plotted in SOCPROG 2.7 [41] and Gephi 0.9.2 [42].

Signature Whistle Identification

Initially, spectrograms (fast Fourier transform (FFT) length 1024, Blackman-Harris window) were inspected in Adobe Audition CC v. 2017.0.2 (Adobe Systems) for instances of signature whistle production. Signature whistles are highly stereotyped and often produced in repetitive sequences [21]. Frequency contours were then extracted from each whistle spectrogram (1024 FFT, overlap 87.5%, Hanning window, time resolution of 1.333 ms) in MATLAB using a supervised contour extraction program [54], with a time resolution of 10 ms. Contour files were then categorised according to their frequency modulation pattern using an automated adaptive resonance theory neural network that incorporates dynamic time warping; ARTwarp [54]. ARTwarp categorises contours based on a set degree of similarity, also known as the vigilance parameter, which, in this instance, was set to 91, as per previous studies [55]. This approach allowed individual signature whistle types to be objectively grouped together in the same category [54], and the ARTwarp analysis was conducted separately for each focal follow.

Each whistle type category was then confirmed as a signature whistle using the SIGID method [21], which uses the temporal patterning that is unique to signature whistles to identify them in free-ranging animals. Whistles were confirmed as signature whistles if the ARTwarp category had at least four whistles in it, and at least once in the sequential bout analysis, 75% or more of those whistles occurred within 1–10 s of one other whistle in that same category [21]. Additionally, each signature whistle also had to be either (1) localized to an individual male that was >±30° from any other individual at least once to confirm identity, and/or (2) confirmed by exclusion where all other signature whistles within the first-order alliance had been localized to an individual male.

Acoustic Similarity

Visual Classification

Visual classification was used to determine signature whistle similarity between allied males, as per previous studies [7, 20, 56, 57]. A signature whistle template from each male was chosen at random, and all whistle templates were plotted as spectrograms with standardized time and frequency axes (scales not plotted). A total of 12 human judges (blind to context and animal identity) were individually asked to rate the similarity of pairs of signature whistles (190 combinations in total), using a five-point similarity index ranging from 1 (least similar) to 5 (most similar) [7, 20, 56, 57]. Template whistles did not change configuration between judges, but the order of slide presentation was randomized in order to eliminate presentation bias. A weighted Cohen's kappa statistic was used to quantify agreement between pairs of judges, which accounts for the degree of disagreement between the judge's ordinal scores [58]. Median similarity scores are provided in Table S1.

Dynamic Time Warp Analysis

Additionally to visual classification, a dynamic time warp approach [22] was used to quantify more subtle differences in whistle similarities based on the shape of the fundamental frequency contour [59]. Ten signature whistle templates were chosen for each male.

Signature whistles often include multiple repetitions of nearly identical loops [60], and the focus of this analysis was to quantify fine-scale similarity between individual signature whistle loops. The fundamental frequency contour was extracted in MATLAB by calculating a spectrogram (24 Hz spectral resolution, 10 ms temporal resolution, 50% overlap, 40 dB dynamic range), and then using a manually supervised ridge tracker to detect and store local peaks in the spectrogram. Gaps were inserted manually between loop repetitions to facilitate isolating individual loops during analysis. To calculate a dissimilarity score, the fundamental frequency contour of each loop was isolated, and the mean fundamental frequency subtracted to account for frequency generalization [61]. A non-Euclidean dynamic time warp distance was calculated using a standard, dynamic time warp algorithm that allowed for unrestricted temporal extension or compression at each time point. To compare whistles with a different number of loops, we calculated an average dissimilarity metric for each pair of whistles by taking the mean dynamic time warp distance across all possible combinations with a single loop from each whistle. Finally, we calculated the dissimilarity between each pair of bottlenose dolphin males as the mean dynamic time warp (DTW) distance across all 10 signature whistles (Table S2).

Dyadic Genetic Relatedness

Genetic data were obtained from small tissue biopsy samples, which were previously collected as part of our long-term research program using a remote biopsy system [62]. Dyadic genetic relatedness between all males in this study was calculated using polymorphic single nucleotide polymorphisms (SNPs) generated by a double digest restriction site associated DNA sequencing (ddRAD) approach [63]. Quality filtered reads were aligned against a *T. truncatus* reference assembly obtained from the NCBI RefSeq database (GenBank: GCA_001922835.1 [47]). Alignment against the reference assembly was done using *bowtie2* version 2.2.6 with the 'very-sensitive' preset. A 'variant-only' v*cf.* file was produced using *HaplotypeCaller* from the Genome Analysis Toolkit GATK version 3.7-0 [43, 44], resulting in 302,012 raw variant calls. Based on call quality (phred quality score > 30), sequencing depth (each locus sequenced at least five times), missing individuals (> 70% individuals covered), and minimal distance between each SNP of at least 100kb, we identified 3,396 high-quality biallelic SNPs per individual [45]. We then used the software Coancestry V1.0.1.5 [46] to estimate pairwise relatedness between individuals using the triadic maximum likelihood estimator [64] (Table S3).

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical procedures were conducted in R 3.3.2 (R project for statistical computing; GNU project). To determine whether or not allied males tended to have signature whistles more similar to each other we conducted two different analyses. First, we ran a cumulative link mixed model (clmm using *ordinal* package in R) on the pairwise median similarity scores as determined by the human judges. Model predictors were pairwise COAs (calculated over a 10 year period) and pairwise relatedness (using polymorphic SNPs). To control for repeated-measures of individuals, individual IDs were included as random effects. The full model was compared to nested models, and a null model containing only the random effects. Model selection was performed by ranking them using Bayesian Information Criterion (BIC), log-likelihood (logLik), and Akaike's Information Criterion (AIC), where the model with the best fit had the lowest aforementioned criterion values. However, models within two AIC units can be considered comparable (Data S1). We also employed anova using the *car* package in R to test whether the inclusion of different parameters in the model explained significantly more variance (Data S1). Second, we ran a linear mixed-effect model fit by REML (lmer using *lme4* package in R) on the mean pairwise dissimilarity scores calculated from the DTW distance. Dissimilarity scores were log transformed (log10) to better fit with an additive variance model. Model predictors and model selection were all as per the analysis conducted on the human judge scores (Data S2). R^2 values for linear mixed-effect models were calculated using the *r.squaredGLMM* function (*MuMIn* package in R) [65]. Finally, to check for correlation between the two signature whistle dissimilarity matrices, we conducted a Mantel test (mantel using *vegan* package in R). The pairwise median similarity scores, as determined by the human judges, were first converted to dissimilarity scores (D) where D = 1-(similarity score-1)/4. The mantel statistic was based on Pearson's product-moment correlation and was calculated using 10,000 permutations [66]. There was significant correlation between the dissimilarity matrices produced by the two different analytical techniques (Mantel test $r = 0.38$, $p = < 0.0001$), revealing some agreement between the two approaches.